PH-7345-A

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CRF RECEPTOR ANTAGONISTS AND METHODS RELATING THERETO

CROSS REFERENCE

This application is a continuation of international application number PCT/US98/02932, filed 17 February 1998.

TECHNICAL FIELD

This invention relates generally to CRF receptor antagonists, and to methods of treating disorders by administration of such antagonists to a warm-blooded animal in need thereof.

BACKGROUND OF THE INVENTION

The first corticotropin-releasing factor (CRF) was isolated from ovine hypothalmi and identified as a 41-amino acid peptide (Vale et al., *Science 213*:1394-1397, 1981). Subsequently, sequences of human and rat CRF were isolated and determined to be identical, but different from ovine CRF in 7 of the 41 amino acid residues (Rivier et al., *Proc. Natl. Acad. Sci. USA 80*:4851, 1983; Shibahara et al., *EMBO J. 2*:775, 1983).

CRF has been found to produce profound alterations in endocrine, nervous and immune system function. CRF is believed to be the major physiological regulator of the basal and stress-release of adrenocorticotropic hormone ("ACTH"), ß-endorphin, and other pro-opiomelanocortin ("POMC")-derived peptides from the anterior pituitary (Vale et al., *Science 213*:1394-1397, 1981). Briefly, CRF is believed to initiate its biological effects by binding to a plasma membrane receptor which has been found to be distributed throughout the brain (DeSouza et al., *Science 224*:1449-1451, 1984), pituitary (DeSouza et al., *Methods Enzymol. 124*:560, 1986; Wynn et al., *Biochem. Biophys. Res. Comm. 110*:602-608, 1983), adrenals (Udelsman et al., *Nature 319*:147-150, 1986) and spleen (Webster, E.L., and E.B. DeSouza, *Endocrinology 122*:609-617, 1988). The CRF receptor is coupled to a GTP-binding protein (Perrin

et al., *Endocrinology 118*:1171-1179, 1986) which mediates CRF-stimulated increase in intracellular production of cAMP (Bilezikjian, L.M., and W.W. Vale, *Endocrinology 113*:657-662, 1983). The receptor for CRF has now been cloned from rat (Perrin et al., *Endo 133*(6):3058-3061, 1993), and human brain (Chen et al., *PNAS 90*(19):8967-8971, 1993; Vita et al., *FEBS 335*(1):1-5, 1993). This receptor is a 415 amino acid protein comprising seven membrane spanning domains. A comparison of identity between rat and human sequences shows a high degree of homology (97%) at the amino acid level.

In addition to its role in stimulating the production of ACTH and POMC, CRF is also believed to coordinate many of the endocrine, autonomic, and behavioral responses to stress, and may be involved in the pathophysiology of affective disorders. Moreover, CRF is believed to be a key intermediary in communication between the immune, central nervous, endocrine and cardiovascular systems (Crofford et al., *J. Clin. Invest. 90*:2555-2564, 1992; Sapolsky et al., *Science 238*:522-524, 1987; Tilders et al., *Regul. Peptides 5*:77-84, 1982). Overall, CRF appears to be one of the pivotal central nervous system neurotransmitters and plays a crucial role in integrating the body's overall response to stress.

Administration of CRF directly to the brain elicits behavioral, physiological, and endocrine responses identical to those observed for an animal exposed to a stressful environment. For example, intracerebroventricular injection of CRF results in behavioral activation (Sutton et al., *Nature 297*:331, 1982), persistent activation of the electroencephalogram (Ehlers et al., *Brain Res. 278*:332, 1983), stimulation of the sympathoadrenomedullary pathway (Brown et al., *Endocrinology 110*:928, 1982), an increase of heart rate and blood pressure (Fisher et al., *Endocrinology 110*:2222, 1982), an increase in oxygen consumption (Brown et al., *Life Sciences 30*:207, 1982), alteration of gastrointestinal activity (Williams et al., *Am. J. Physiol. 253*:G582, 1987), suppression of food consumption (Levine et al., *Neuropharmacology 22*:337, 1983), modification of sexual behavior (Sirinathsinghji et al., *Nature 305*:232, 1983), and immune function compromise (Irwin et al., *Am. J. Physiol. 255*:R744, 1988). Furthermore, clinical data suggests that CRF may be hypersecreted in the brain in depression, anxiety-related disorders, and anorexia

nervosa. (DeSouza, *Ann. Reports in Med. Chem.* *25*:215-223, 1990). Accordingly, clinical data suggests that CRF receptor antagonists may represent novel antidepressant and/or anxiolytic drugs that may be useful in the treatment of the neuropsychiatric disorders manifesting hypersecretion of CRF.

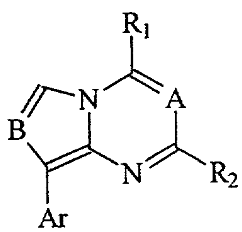
The first CRF receptor antagonists were peptides (*see, e.g.*, Rivier et al., U.S. Patent No. 4,605,642; Rivier et al., *Science 224*:889, 1984). While these peptides established that CRF receptor antagonists can attenuate the pharmacological responses to CRF, peptide CRF receptor antagonists suffer from the usual drawbacks of peptide therapeutics including lack of stability and limited oral activity. More recently, small molecule CRF receptor antagonists have been reported. For example, substituted 4-thio-5-oxo-3-pyyrazoline derivatives (Abreu et al., U.S. Patent No. 5,063,245) and substituted 2-aminothiazole derivatives (Courtemanche et al., Australian Patent No. AU-A-41399/93) have been reported as CRF receptor antagonists. These particular derivatives were found to be effective in inhibiting the binding of CRF to its receptor in the 1-10 μM range and 0.1-10 μM range, respectively.

Due to the physiological significance of CRF, the development of biologically-active small molecules having significant CRF receptor binding activity and which are capable of antagonizing the CRF receptor remains a desirable goal. Such CRF receptor antagonists would be useful in the treatment of endocrine, psychiatric and neurologic conditions or illnesses, including stress-related disorders in general.

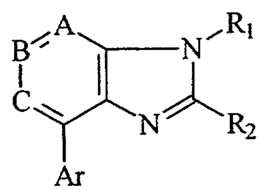
While significant strides have been made toward achieving CRF regulation through administration of CRF receptor antagonists, there remains a need in the art for effective small molecule CRF receptor antagonists. There is also a need for pharmaceutical compositions containing such CRF receptor antagonists, as well as methods relating to the use thereof to treat, for example, stress-related disorders. The present invention fulfills these needs, and provides other related advantages.

SUMMARY OF THE INVENTION

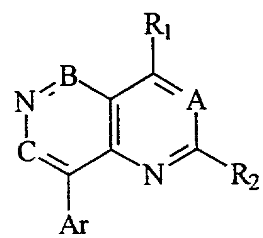
In brief, this invention is generally directed to CRF receptor antagonists, and more specifically to CRF receptor antagonists having the following general structures (I) through (VI):



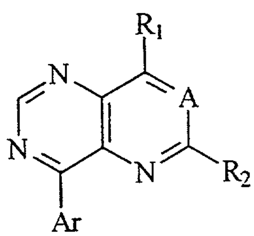
(I)



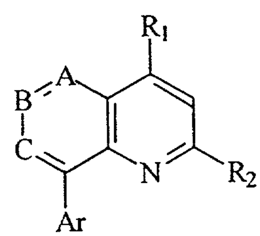
(II)



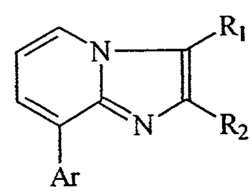
(III)



(IV)



(V)



(VI)

where A, B, C, Ar, R_1 and R_2 are as identified in the following detailed description.

The CRF receptor antagonists of this invention have utility over a wide range of therapeutic applications, and may be used to treat a variety of disorders or illnesses, including stress-related disorders. Such methods include administering an effective amount of a CRF receptor antagonist of this invention, preferably in the form of a pharmaceutical composition, to an animal in need thereof. Accordingly, in another embodiment, pharmaceutical compositions are disclosed containing one or more CRF receptor antagonists of this invention in combination with a pharmaceutically acceptable carrier and/or diluent.

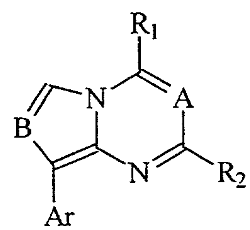
These and other aspects of the invention will be apparent upon reference to the following detailed description. To this end, various references are set forth herein

which describe in more detail certain procedures, compounds and/or compositions, and are hereby incorporated by reference in their entirety.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed generally to compounds useful as corticotropin-releasing factor (CRF) receptor antagonists.

In a first embodiment, the CRF receptor antagonists of this invention have the following structure (I):



(I)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

A and B are selected from CR and N;

R is selected from hydrogen and C_{1-6}alkyl;

R_1 is NR_3R_4;

R_2 is C_{1-6}alkyl;

R_3 is selected from hydrogen, C_{1-6}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, C_{3-6}cycloalkyl; C_{3-6}alkenyl; hydroxyC_{1-6}alkyl, C_{1-6}alkylcarbonyloxyC_{1-6}alkyl and C_{1-6}alkyloxyC_{1-6}alkyl;

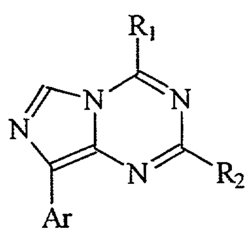
R_4 is selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

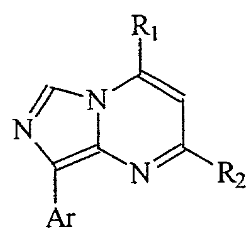
Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, triflouromethyl and C_{1-6}alkyl substituted with morpholinyl.

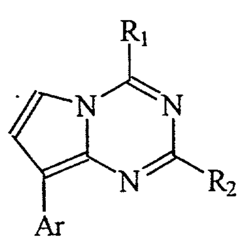
According, the CRF receptor antagonists of this embodiment have one of the following structures (Ia), (Ib), (Ic) and (Id):



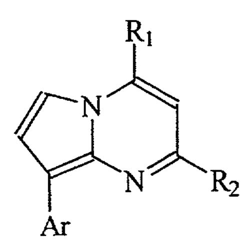
(Ia)



(Ib)

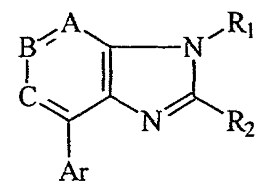


(Ic)



(Id)

In a second embodiment, the CRF receptor antagonists of this invention have the following structure (II):



(II)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

A, B and C are selected from CR and N, with the proviso that when B is N both A and C are CR;

R is selected from hydrogen and C_{1-6}alkyl;

R_1 is selected from NR_3R_4 and R_5;

R_2 is C_{1-6}alkyl;

R_3 is selected from hydrogen, C_{1-6}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, C_{3-6}cycloalkyl; C_{3-6}alkenyl; hydroxyC_{1-6}alkyl, C_{1-6}alkylcarbonyloxyC_{1-6}alkyl and C_{1-6}alkyloxyC_{1-6}alkyl;

R_4 and R_5 are independently selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

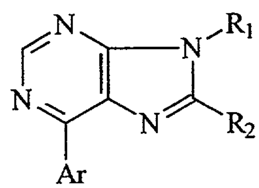
or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl,

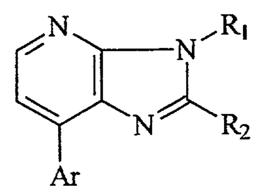
triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, triflouromethyl and C_{1-6}alkyl substituted with morpholinyl.

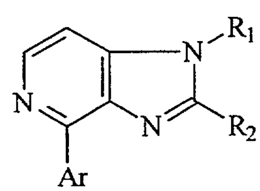
According, the CRF receptor antagonists of this embodiment have one of the following structures (IIa), (IIb), (IIc), (IId) and (IIe):



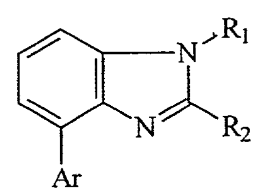
(IIa)



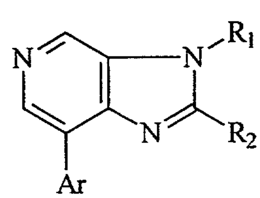
(IIb)



(IIc)

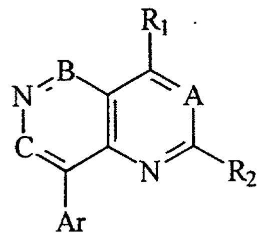


(IId)



(IIe)

In a third embodiment, the CRF receptor antagonists of this invention have the following structure (III):



(III)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

A, B and C are selected from CR and N, with the proviso that one, and only one, of B and C is N;

R is selected from hydrogen and C_{1-6}alkyl;

R_1 is NR_3R_4;

R_2 is C_{1-6}alkyl;

R_3 is selected from hydrogen, C_{1-6}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, C_{3-6}cycloalkyl; C_{3-6}alkenyl; hydroxyC_{1-6}alkyl, C_{1-6}alkylcarbonyloxyC_{1-6}alkyl and C_{1-6}alkyloxyC_{1-6}alkyl;

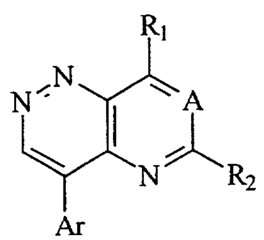
R_4 is selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

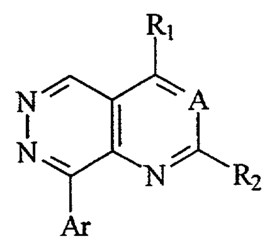
Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$alkyloxy, di($C_{1\text{-}6}$alkyl)amino$C_{1\text{-}6}$alkyl, triflouromethyl and $C_{1\text{-}6}$alkyl substituted with morpholinyl.

According, the CRF receptor antagonists of this embodiment have one of the following structures (IIIa) and (IIIb):

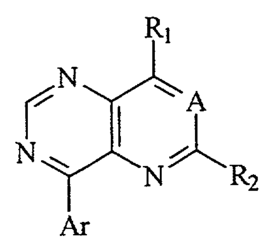


(IIIa)



(IIIb)

In a fourth embodiment, the CRF receptor antagonists of this invention have the following structure (IV):



(IV)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

A is selected from CR and N;

R is selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_1 is NR_3R_4;

R_2 is $C_{1\text{-}6}$alkyl;

R_3 is selected from hydrogen, $C_{1\text{-}6}$alkyl, mono- or di($C_{3\text{-}6}$cycloalkyl)methyl, $C_{3\text{-}6}$cycloalkyl; $C_{3\text{-}6}$alkenyl; hydroxy$C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$alkylcarbonyloxy$C_{1\text{-}6}$alkyl and $C_{1\text{-}6}$alkyloxy$C_{1\text{-}6}$alkyl;

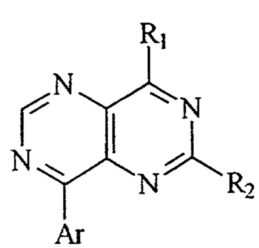
R_4 is selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

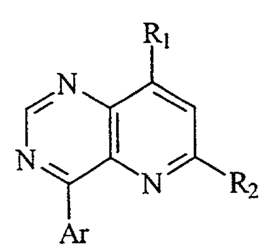
Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy. C_{1-6}alkylthio. nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, triflouromethyl and C_{1-6}alkyl substituted with morpholinyl.

According, the CRF receptor antagonists of the this embodiment have one of the following structures (IVa) and (IVb):

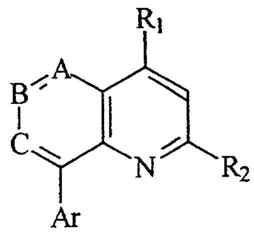


(IVa)



(IVb)

In a fifth embodiment, the CRF receptor antagonists of this invention have the following structure (V):



(V)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

A, B and C are selected from CR and N, with the proviso that one, and only one, of A, B and C is N;

R is selected from hydrogen and C_{1-6}alkyl;

R_1 is NR_3R_4;

R_2 is C_{1-6}alkyl;

R_3 is selected from hydrogen, C_{1-6}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, C_{3-6}cycloalkyl; C_{3-6}alkenyl; hydroxyC_{1-6}alkyl, C_{1-6}alkylcarbonyloxyC_{1-6}alkyl and C_{1-6}alkyloxyC_{1-6}alkyl;

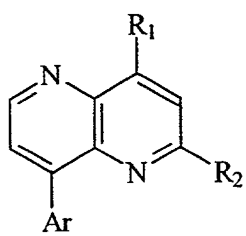
R_4 is selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

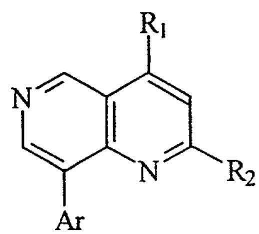
Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, triflouromethyl and C_{1-6}alkyl substituted with morpholinyl.

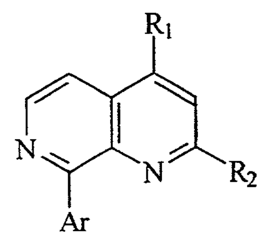
According, the CRF receptor antagonists of this embodiment have one of the following structures (Va), (Vb) and (Vc):



(Va)

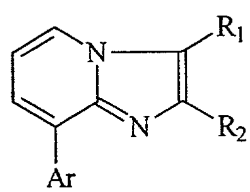


(Vb)



(Vc)

In a sixth embodiment, the CRF receptor antagonists of this invention have the following structure (VI):



(VI)

including stereoisomers and pharmaceutically acceptable salts thereof, wherein:

R_1 is selected from NR_3R_4 and R_5;

R_2 is C_{1-6}alkyl;

R_3 is selected from hydrogen, C_{1-6}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, C_{3-6}cycloalkyl; C_{3-6}alkenyl; hydroxyC_{1-6}alkyl, C_{1-6}alkylcarbonyloxyC_{1-6}alkyl and C_{1-6}alkyloxyC_{1-6}alkyl;

R_4 and R_5 are independently selected from C_{1-8}alkyl, mono- or di(C_{3-6}cycloalkyl)methyl, Ar^1CH_2, C_{3-6}alkenyl, C_{1-6}alkyloxyC_{1-6}alkyl, hydroxyC_{1-6}alkyl, thienylmethyl, furanylmethyl, C_{1-6}alkylthioC_{1-6}alkyl, morpholinyl, mono- or di(C_{1-}

$_6$alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)amino, C_{1-6}alkylcarbonylC_{1-6}alkyl, C_{1-6}alkyl substituted with imidazolyl; or a radical of the formula -(C_{1-6}alkanediyl)-O-CO-Ar^1;

or R_3 and R_4 taken together with the nitrogen atom to which they are attached form a pyrrolidinyl, piperidinyl, homopiperidinyl or morpholinyl group, optionally substituted with C_{1-6}alkyl or C_{1-6}alkyloxy;

Ar is selected from phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino and mono- and di(C_{1-6}alkyl)amino; and pyridinyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, triflouromethyl, hydroxy, cyano, C_{1-6}alkyloxy, benzyloxy, C_{1-6}alkylthio, nitro, amino, mono- and di(C_{1-6}alkyl)amino and piperidinyl; and

Ar^1 is selected from phenyl, pyridinyl, and phenyl substituted with 1, 2 or 3 substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, triflouromethyl and C_{1-6}alkyl substituted with morpholinyl.

The compounds of the present invention may be prepared by known organic synthesis techniques, including the methods described in more detail in the Examples.

The compounds of the present invention may generally be utilized as the free base. Alternatively, the compounds of this invention may be used in the form of acid addition salts. Acid addition salts of the free base amino compounds of the present invention may be prepared by methods well known in the art, and may be formed from organic and inorganic acids. Suitable organic acids include maleic, fumaric, benzoic, ascorbic, succinic, methanesulfonic, acetic, oxalic, propionic, tartaric, salicylic, citric, gluconic, lactic, mandelic, cinnamic, aspartic, stearic, palmitic, glycolic, glutamic, and benzenesulfonic acids. Suitable inorganic acids include hydrochloric, hydrobromic, sulfuric, phosphoric, and nitric acids.

The effectiveness of a compound as a CRF receptor antagonist may be determined by various assay methods. Suitable CRF antagonists of this invention are capable of inhibiting the specific binding of CRF to its receptor and antagonizing activities associated with CRF. A compound of structure (I) through (VI) may be

assessed for activity as a CRF antagonist by one or more generally accepted assays for this purpose, including (but not limited to) the assays disclosed by DeSouza et al. (*J. Neuroscience* *7*:88, 1987) and Battaglia et al. (*Synapse* *1*:572, 1987). As mentioned above, suitable CRF antagonists include compounds which demonstrate CRF receptor affinity. CRF receptor affinity may be determined by binding studies that measure the ability of a compound to inhibit the binding of a radiolabeled CRF (*e.g.*, [^{125}I]tyrosine-CFR) to its receptor (*e.g.*, receptors prepared from rat cerebral cortex membranes). The radioligand binding assay described by DeSouza et al. (*supra*, 1987) provides an assay for determining a compound's affinity for the CRF receptor. Such activity is typically calculated from the IC_{50} as the concentration of a compound necessary to displace 50% of the radiolabeled ligand from the receptor, and is reported as a "K_i" value calculated by the following equation:

$$K_i = \frac{IC_{50}}{1 + L / K_D}$$

where L = radioligand and K_D = affinity of radioligand for receptor (Cheng and Prusoff, *Biochem. Pharmacol.* *22*:3099, 1973).

In addition to inhibiting CRF receptor binding, a compound's CRF receptor antagonist activity may be established by the ability of the compound to antagonize an activity associated with CRF. For example, CRF is known to stimulate various biochemical processes, including adenylate cyclase activity. Therefore, compounds may be evaluated as CRF antagonists by their ability to antagonize CRF-stimulated adenylate cyclase activity by, for example, measuring cAMP levels. The CRF-stimulated adenylate cyclase activity assay described by Battaglia et al. (*supra*, 1987) provides an assay for determining a compound's ability to antagonize CRF activity. Accordingly, CRF receptor antagonist activity may be determined by assay techniques which generally include an initial binding assay (such as disclosed by DeSouza (*supra*, 1987)) followed by a cAMP screening protocol (such as disclosed by Battaglia (*supra*, 1987)).

With reference to CRF receptor binding affinities, CRF receptor antagonists of this invention have a K_i of less than 10 μM. In a preferred embodiment of this invention, a CRF receptor antagonist has a K_i of less than 1μM, and more preferably less than 0.25 μM (*i.e.*, 250 nM).

The CRF receptor antagonists of the present invention demonstrate activity at the CRF receptor site, and may be used as therapeutic agents for the treatment of a wide range of disorders or illnesses including endocrine, psychiatric, and neurologic disorders or illnesses. More specifically, the CRF receptor antagonists of the present invention may be useful in treating physiological conditions or disorders arising from the hypersecretion of CRF. Because CRF is believed to be a pivotal neurotransmitter that activates and coordinates the endocrine, behavioral and automatic responses to stress, the CRF receptor antagonists of the present invention can be used to treat neuropsychiatric disorders. Neuropsychiatric disorders which may be treatable by the CRF receptor antagonists of this invention include affective disorders such as depression; anxiety-related disorders such as generalized anxiety disorder, panic disorder, obsessive-compulsive disorder, abnormal aggression, cardiovascular abnormalities such as unstable angina and reactive hypertension; and feeding disorders such as anorexia nervosa, bulimia, and irritable bowel syndrome. CRF antagonists may also be useful in treating stress-induced immune suppression associated with various diseases states, as well as stroke. Other uses of the CRF antagonists of this invention include treatment of inflammatory conditions (such as rheumatoid arthritis, uveitis, asthma, inflammatory bowel disease and G.I. motility), Cushing's disease, infantile spasms, epilepsy and other seizures in both infants and adults, and various substance abuse and withdrawal (including alcoholism).

In another embodiment of the invention, pharmaceutical compositions containing one or more CRF receptor antagonists are disclosed. For the purposes of administration, the compounds of the present invention may be formulated as pharmaceutical compositions. Pharmaceutical compositions of the present invention comprise a CRF receptor antagonist of the present invention (*i.e.*, a compound of structure (I) through (VI)) and a pharmaceutically acceptable carrier and/or diluent. The

CRF receptor antagonist is present in the composition in an amount which is effective to treat a particular disorder--that is, in an amount sufficient to achieve CRF receptor antagonist activity, and preferably with acceptable toxicity to the patient. Preferably, the pharmaceutical compositions of the present invention may include a CRF receptor antagonist in an amount from 0.1 mg to 250 mg per dosage depending upon the route of administration, and more preferably from 1 mg to 60 mg. Appropriate concentrations and dosages can be readily determined by one skilled in the art.

Pharmaceutically acceptable carrier and/or diluents are familiar to those skilled in the art. For compositions formulated as liquid solutions, acceptable carriers and/or diluents include saline and sterile water, and may optionally include antioxidants, buffers, bacteriostats and other common additives. The compositions can also be formulated as pills, capsules, granules, or tablets which contain, in addition to a CRF receptor antagonist, diluents, dispersing and surface active agents, binders, and lubricants. One skilled in this art may further formulate the CRF receptor antagonist in an appropriate manner, and in accordance with accepted practices, such as those disclosed in *Remington's Pharmaceutical Sciences*, Gennaro, Ed., Mack Publishing Co., Easton, PA 1990.

In another embodiment, the present invention provides a method for treating a variety of disorders or illnesses, including endocrine, psychiatric and neurologic disorders or illnesses. Such methods include administering of a compound of the present invention to a warm-blooded animal in an amount sufficient to treat the disorder or illness. Such methods include systemic administration of a CRF receptor antagonist of this invention, preferably in the form of a pharmaceutical composition. As used herein, systemic administration includes oral and parenteral methods of administration. For oral administration, suitable pharmaceutical compositions of CRF receptor antagonists include powders, granules, pills, tablets, and capsules as well as liquids, syrups, suspensions, and emulsions. These compositions may also include flavorants, preservatives, suspending, thickening and emulsifying agents, and other pharmaceutically acceptable additives. For parental administration, the compounds of the present invention can be prepared in aqueous injection solutions which may contain,

in addition to the CRF receptor antagonist, buffers, antioxidants, bacteriostats, and other additives commonly employed in such solutions.

As mentioned above, administration of a compound of the present invention can be used to treat a wide variety of disorders or illnesses. In particular, the compounds of the present invention may be administered to a warm-blooded animal for the treatment of depression, anxiety disorder, panic disorder, obsessive-compulsive disorder, abnormal aggression, unstable angina, reactive hypertension, anorexia nervosa, bulimia, irritable bowel syndrome, stress-induced immune suppression, stroke, inflammation, Cushing's disease, infantile spasms, epilepsy, and substance abuse or withdrawal.

The following examples are provided for purposes of illustration, not limitation.

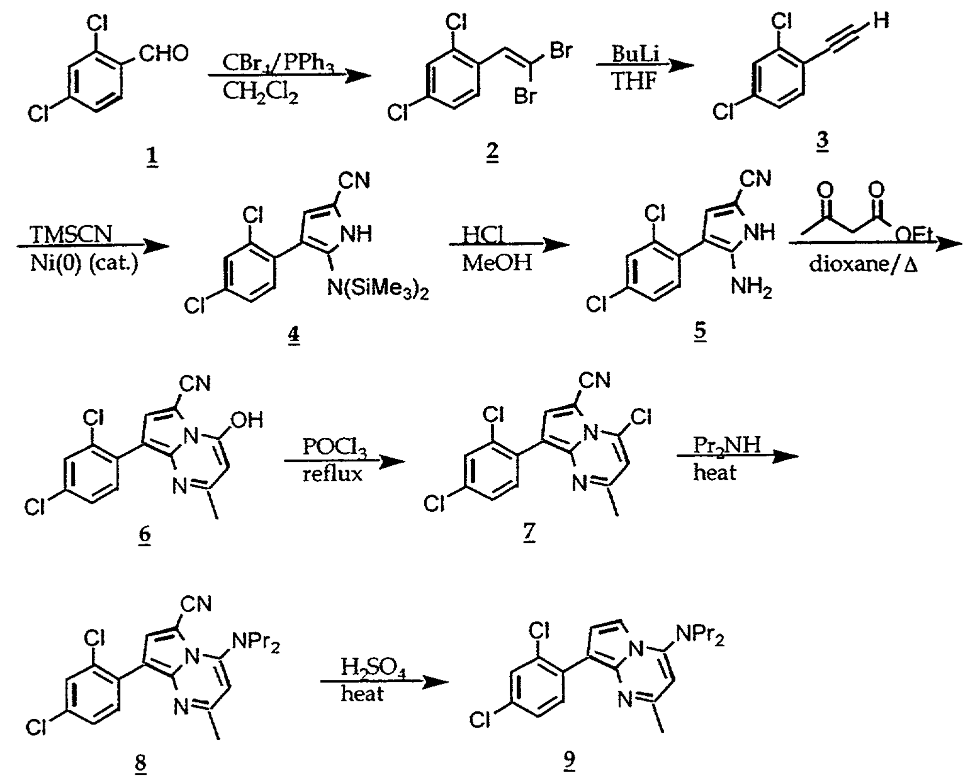
EXAMPLES

The CRF receptor antagonists of this invention may be prepared by the methods disclosed in Examples 1-19. Example 20 presents a method for determining the receptor binding activity (K_i), and Example 21 discloses an assay for screening compounds of this invention for CRF-stimulated adenylate cyclase activity.

EXAMPLE 1

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (I)

Part A. Pyrrolo[1,2-a]pyrimidines



1,1-Dibromo-2-(2,4-dichlorophenyl)ethene (2)

Into a solution of 2,4-dichlorobenzaldehyde (8.7 g, 50 mmol) and carbon tetrabromide (18.3 g, 55 mmol) in dichloromethane (200 ml) was added portionwise triphenylphosphine (28.8 g, 110 mmol) at 0°C. The slightly yellow mixture was stirred at room temperature for 1 hour and diluted with hexanes (800 ml). This mixture was then filtrated through a short silica gel column with 1:10 ethyl acetate-hexanes and the filtrate was concentrated *in vacuo* to give a white solid (16.5 g, 100%). recrystallization from ether-hexanes gave a white crystalline product (14.2 g, 86% yield); ^{1}H NMR ($TMS/CDCl_3$):

2,4-Dichlorophenylacetylene (3)

A solution of 1,1-dibromo-2-(2,4-dichlorophenyl)ethene (14 g, 42.4 mmol) in THF (100 ml) at -78°C under nitrogen was treated with butyllithium (1.6 M solution in hexane, 28 ml, 44.8 mmol). after being stirred for 1 hour at -78°C, the reaction mixture was warmed to room temperature and stirred for another hour. The reaction was quenched with water and the product was extracted with hexanes. The extract was dried over $MgSO_4$, filtrated and concentrated *in vacuo* to give the product. (Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* 1972, 3769)

2-Di(trimethylsilyl)amino-3-(2,4-dichlorophenyl)-5-cyanopyrrole (4)

In a 200-ml reaction flask was placed $NiCl_2$ (0.2 g, 1.5 mmol) and then 1N DIBAL-H in hexane (3 ml, 3 mmol) was added. After the color of the catalyst turned to black, Me_3SiCN (30 ml, 0.225 mol.) and 2,4-dichlorophenylacetylene (6.4 g, 37.5 mmol) were added to the reaction flask. The mixture was stirred under reflux for 20 hours. The product was isolated by column chromatography (silica gel, hexanes/EtOAc, 5/1) to afford pure 4 (60% yield). (Chatani, N.; Takeyasu, T, Horiuchi, N; Hanafusa, *J. Org. Chem. 53*:3539, 1988)

2-Amino-3-(2,4-dichlorophenyl)-5-cyanopyrrole (5)

A solution of 2-(di(trimethylsilyl)amino-3-(2,4-dichlorophenyl)-5-cyanopyrrole (4, 11.8 g, 30 mmol) in methanol (50 ml) is treated with 2N aqueous hydrochloric acid (30 ml). The mixture is heated to reflux for 1 hour and concentrated in vacuo. The aqueous phase then is basified with solid sodium carbonate and the product is extracted with ethyl acetate. The extract is washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give the product.

1-Cyano-3-(dichlorophenyl)-5-methyl-7-hydroxypyrrolo[1,2-a]pyrimidine (6)

A solution of 2-amino-3-(2,4-dichlorophenyl)-5-cyanopyrrole (5, 5 g, 20 mmol) and ethyl acetoacetate (5.2 g, 40 mmol) in dioxane (50 ml) is heated to reflux overnight. The cold solution is treated with ether and hexanes and the solid product is collected by vacuo filtration.

1-Cyano-3-(dichlorophenyl)-5-methyl-7-chloropyrrolo[1,2a]pyrimidine (7)

A mixture of 1-cyano-3-(dichlorophenyl)-5-methyl-7-hydroxypyrrolo[1,2-a]pyrimidine (6, 0.64 g, 2 mmol) and $POCl_3$ (3 ml) is heated to reflux for 2 hours. The reaction mixture is hydrolyzed with ice water and the product is extracted with ethyl acetate. The extract was washed with saturated aqueous sodium bicarbonate and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo* to afford the product.

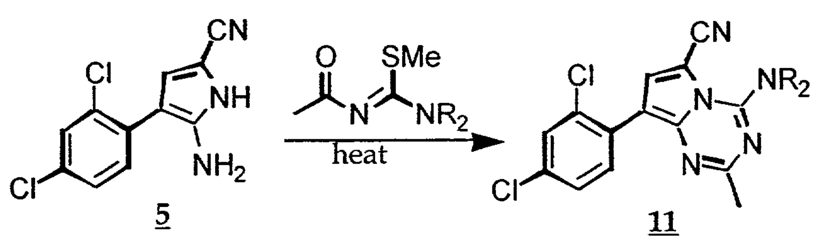
1-Cyano-3-(dichlorophenyl)-5-methyl-7-dipropylaminopyrrolo[1,2-a]pyrimidine (8)

A mixture of 1-cyano-3-(dichlorophenyl)-5-methyl-7-chloropyrrolo[1,2-a]pyrimidine (7, 335 mg, 1 mmol) and dipropylamine (1 ml) was heated at 100°C in a reacti-vial for 2 hours. The product is purified by chromatography on silica gel.

3-(Dichlorophenyl)-5-methyl-7-dipropylaminopyrrolo[1,2-a]pyrimidine (9)

A solution of 1-cyano-3-(dichlorophenyl)-5-methyl-7-dipropylaminopyrrolo[1,2-a]pyrimidine (8, 200 mg, 0.5 mmol) in THF (5 ml) is treated with 2N aqueous H_2SO_4 (2 ml) and the mixture is heated to reflux for 6 hours. The mixture is neutralized with triethylamine and the product is purified by chromatography on silica gel.

Part B. Pyrrolo[1,2-a]-s-triazines



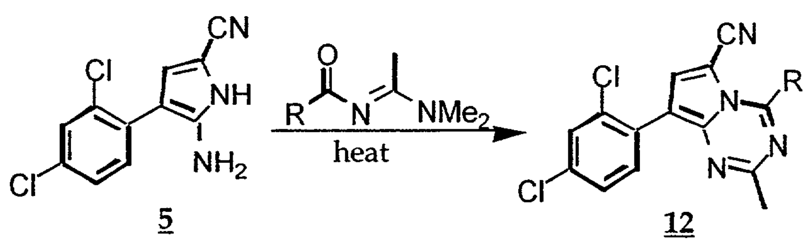
N-Acetyl-N'-propyl-N'-cyclopropanemethyl-S-methylurea (10)

Sodium isothiocyanate (8.9 g, 0.11 mol.) was dissolved in acetone (200 ml) and treated with acetyl chloride (7.8 g, 0.1 mol.) at room temperature. The suspension was stirred at room temperature for 10 minutes before N-propyl-N-

cyclopropanemethylamine (11.3 g, 0.1 mol.) was added. This mixture was stirred at room temperature for 15 minutes and MeI (18.5 g, 0.13 mol.) and Na_2CO_3 (13.8g, 0.11 mol.) were introduced. The mixture was then stirred at room temperature overnight and concentrated in vacuo. The residue was partitioned in ethyl acetate-water. The organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic layer was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give the product as a yellowish oil. ^{1}H NMR: 0.27 (m, 2H), 0.57 m, 2H), 1.08 (m, 1H), 1.68 (m, 2H), 2.17 (s, 3H), 2.38 (s, 3H), 3.39 (d, 2H), 3.51 (t, 2H); MS (ion spray): 229 (M+H).

1-Cyano-3-(dichlorophenyl)-5-methyl-7-dipropylaminopyrrolo[1,2-a]-s-triazine (11)

A mixture of 2-Amino-3-(2,4-dichlorophenyl)-5-cyanopyrrole (5, 0.5 g, 2 mmol) and N,N-dipropyl-N'-acetyl-S-methylthiourea (10, 1.08 g, 5 mmol) is heated at 180°C in a sealed reacti-vial for 16 hours. Chromatography on silica gel affords the designed product.

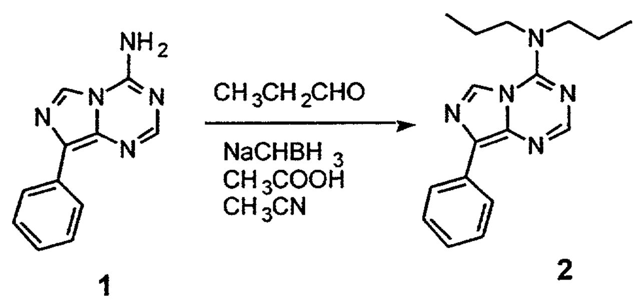


1-Cyano-3-(2,4-dichlorophenyl)-5-methyl-7-(1-ethylpentyl)pyrrolo[1,2-a]-s-triazine (12)

A solution of 2-amino-1-cyano-3-(2,4-dichlorophenyl)-1H-pyrrole (90 mg) and N-dimethyl-N'-(2-ethylhexanoyl)-acetamidine (180 mg) in dioxane (5 ml) was heated to reflux overnight. Chromatography on silica gel with 1:5 ethyl acetate-hexanes gave the title compound.

EXAMPLE 2

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (I)



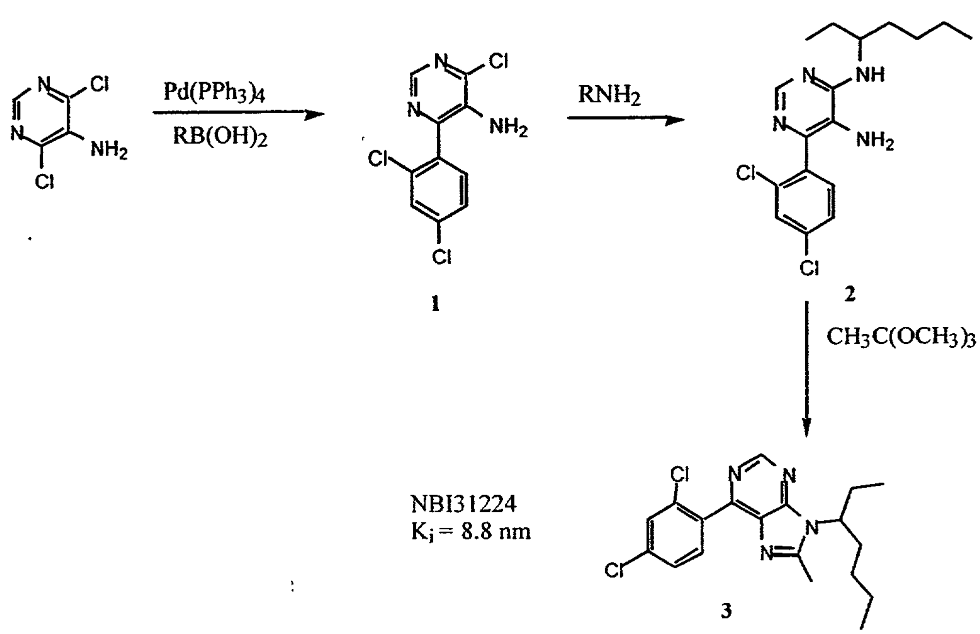
Preparation of 2-Methyl-4-dipropylamino-8-phenyl-imidazo[1,5-a]-1,3,5-triazine (2).

Add sodium cyanoborohydride and then glacial acetic acid (0.1 mL) to a stirring solution of (1) (225 mg, 1 mmol) and propionaldehyde (580 mg, 10 mmol) in acetonitrile (5 mL) at 0°C. Stir the reaction mixture at room temperature for 2 hrs. Patition the reaction mixture between EtOAc and saturated aq. $NaHCO_3$. Wash the EtOAc layer by Brine, dry under $NaSO_4$, filter, concentrate to afford a dark residue, which is purified by flash column on silica gel to yield the desired product (2).

(R. Balicki, R.S. Hosmane and N.J. Leonard, *J. Org. Chem.* *48*:3, 1983; R. Borch and A. Hassid, *J. Org. Chem.* *37*:1673, 1972)

EXAMPLE 3

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (II)



NBI31224
$K_i = 8.8$ nm

Synthesis of Compound 1

A mixture of 5-Amino-4,6-dichloropyrimidine (1.3 gms, 7.92 mmol), 2,4-Dichlorobenzene boranic acid (1.8 gms, 9.5 mmol), tetrakis triphenylphospine palladium (0.95 mmol), and potassium carbonate (2.12 gms) was partitioned in a mixture of toluene, ethanol, and water (35 ml, 10 ml, 10 ml respectively). The mixture was refluxed under a nitrogen atmosphere for 18 hrs. After reflux, the solution was quenched with 50 ml saturated ammonium chloride and extracted with ethyl acetate (2x300 ml). The organic layers were combined, dried over sodium sulfate and concentrated to yield oil. The crude oil was purified with flash chromatography eluting with hexanes:ethyl acetate (5:1) to yield yellow solid. 40% yield. d 4.15 (br, 2H, NH), 7.34 (m, 2H, Ar), 7.53 (s, 1H, Ar), 8.43 (s, 1H, Ar(pyrmidine))ppm.

Synthesis of Compound 2

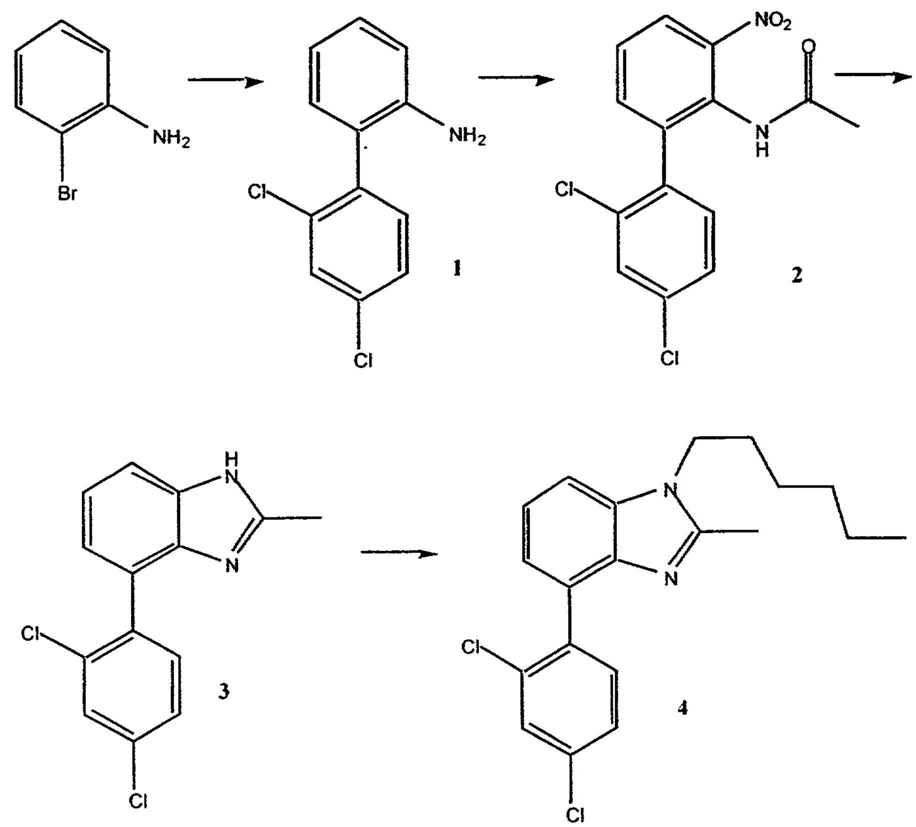
A mixture of compound 1 (250 mgs, 1.0 mmol) and 3-aminoheptane (0.3 ml) were placed in a 1 ml reaction vial and heated to 130°C overnight. After overnight, the reaction was cooled and loaded directly on silica prep plate, eluting with hexanes:ether (5:1). Compound was isolated as a solid. δ 0.98 (m, 5H), 1.35 (m, 5H), 1.6 (m, 4H), 3.05 (br, 2H, NH), 4.2 (m, 1H, CH), 4.85 (d, 1H, NH), 7.32 (s, 2H, Ar), 7.49 (s, 1H, Ar), 8.29 (s, 1H, Ar-pyr) ppm. M+H 367

6-(2,4-Dichlorophenyl)-8-methyl-9-(3-heptanyl)purine (3)

Compound 2 (50 mgs, 0.14 mmol) and triethyl orthoacetate (0.5 ml) were placed in a reaction vial and heated at 110°C for 10 hrs. After reflux, the reaction was partitioned between ethyl acetate (10 ml) and water (10 ml). The organic layer was extracted, dried over sodium sulfate and all solvent removed. Compound was purified on silica prep plate with hexanes:ether (1:5). 7 mgs isolated. δ 0.91 (m, 5H), 1.30 (m, 5H), 1.9 (m, 4H), 2.63 (s, 3H, methyl), 4.3 (m, 1H, CH), 7.41 (d, 2H, Ar), 7.56 (s, 1H, Ar), 7.64 (d,1H, Ar), 8.9 (s, 1H, Ar-pyr) ppm. M+H=377

EXAMPLE 4

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (II)



2-Amino-6,8-dichlorobiphenyl (1)

A stirred solution of 2-bromoaniline (1.0 g, 5.8 mmol) in 30 mL of toluene was treated with tetrakis(triphenylphosphine)palladium(0) (672 mg, 0.58 mmol, 10% mol) and 2.0M aqueous sodium carbonate solution (8.8 mL, 17.4 mmol). This mixture was treated with dichlorobenzeneboronic acid (2.28 g, 11.0 mmol) ethyl alcohol (8.8 mL). The resulting brown mixture was heated to reflux overnight. The reaction mixture was cooled, diluted with ethyl acetate and washed with saturated ammonium chloride solution once. The organic layer was dried by sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on silica gel to provide the desired product (1) (1.0 g, 4.20 mmol, 72%), which was confirmed by

GC/MS and ^{1}H NMR. GC/MS: m/z = 237, 239; 300 MHz ^{1}H NMR ($CDCl_3$): δ 3.54 (br s, 2H), 6.78 (d, 1H), 6.84 (d, 1H), 7.01 (d, 1H), 7.19-7.35 (m, 3H), 7.53 (d, 1H).

2-Acetamido-3-nitro-6,8-dichlorobiphenyl (2)

A solution of 2-amino-6,8-dichlorobiphenyl (1) (0.5g, 2.1 mmol) in 0.5 mL AcOH and 1 mL Ac_2O was heated to 100°C for 15 min., then allowed to come to room temperature. This solution is treated with 90% fuming nitric acid (0.5 mL) and stirred at 5°C then allowed to warm to room temperature. After the starting material is mostly consumed, the solution is poured into water and the crude product is isolated by extraction with ethyl acetate. Flash chromatography using silica gel and ethyl acetate/hexane gives the desired title compound.

1-Methyl-4-(2',4'-dichlorophenyl)benzimidazole (3)

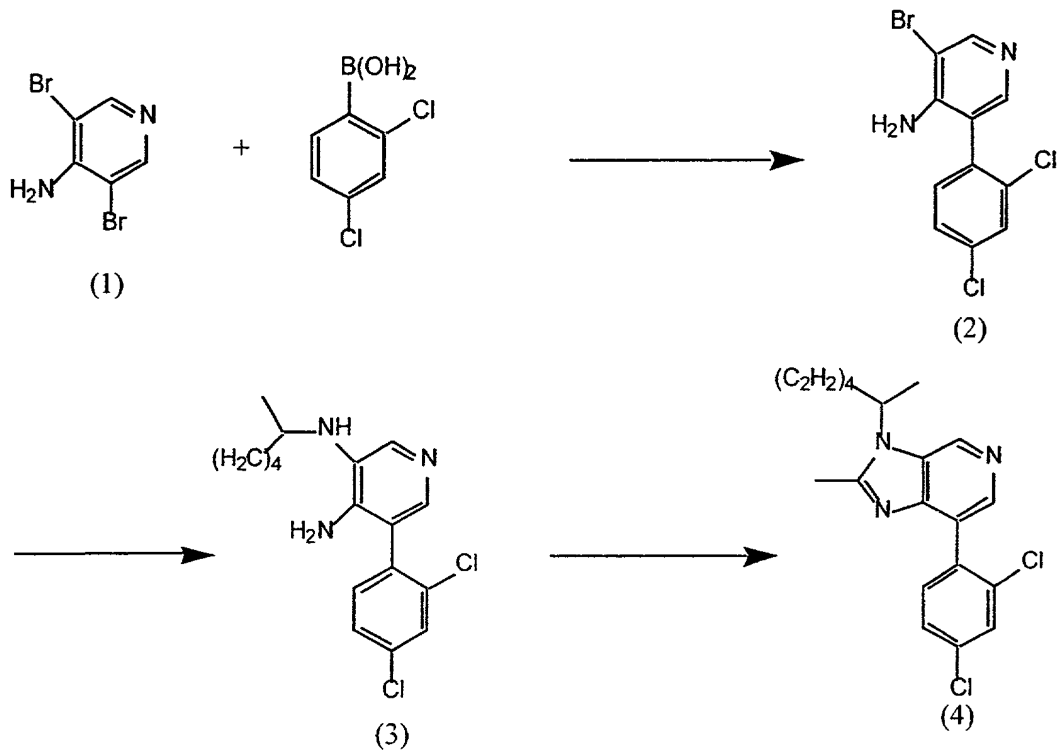
A solution of 2-Acetamido-3-nitro-6,8-dichlorobiphenyl (2) (2.1 mmol) in 10 mL AcOH, is treated with 100 mg of 10% Pd/C and shaken under an atmosphere of hydrogen until the starting material is ca. 95% consumed. The mixture is filtered then heated to 100°C until the cyclization is ca. 90% complete, then allowed to come to room temperature. This solution is poured into water and the title product isolated by filtration. This product is then purified by flash chromatography on silica using ethyl acetate/hexanes.

1-Methyl-4-(2',4'-dichlorophenyl)-N-hexylbenzimidazole (4)

A solution of 1-methyl-4-(2',4'-dichlorophenyl)benzimidazole (3) (1 mmol) in 1 mL DMF, is treated with 300 mg of hexyl chloride and cesium chloride (2 mmole) then stirred under nitrogen. After 16 hours this solution is poured into water and the product isolated by extraction with ethyl acetate. The title compound is then purified by flash chromatography on silica, using ethyl acetate/hexanes.

EXAMPLE 5

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (II)



3-bromo-5-(3,4-dichlorophenyl)-4-amino-pyridine (2):

A solution of 3,5-dibromo-4-amino-pyridine 1 (4.0 g,15.87 mmol), $Pd(PPh_3)_4$ (0.91g, 0.78 mmol) and aqueous solution of Na_2CO_3 (23.8 ml, 2M) in toluene (80 ml) is added to a solution of dichlorobenzene boronic acid (6.23 g, 46.4 mmole) in ethanol (24 ml). The mixture is refluxed for 14 h., diluted with ethyl acetate and washed with saturated NH_4Cl solution. The organic layer is dried over sodium sulfate and concentrated in *vacuo*. The residue is chromatographed on silica gel to give 2.

<u>5-(3,4-dichlorophenyl)-3-aminoheptane-4-amino-pyridine (3):</u>

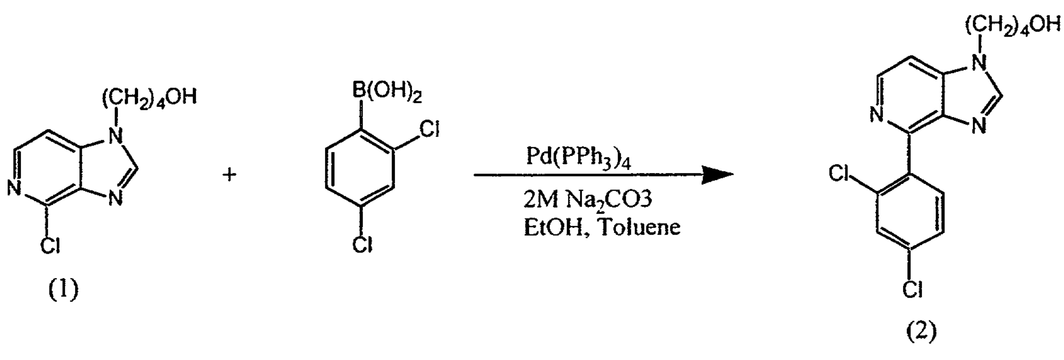
A mixture of 2 (1 mmol), 3-aminoheptane (1.2 mmol), $Pd_2(DBA)_3$ (0.02 mmol, 4 mol % Pd, 18 mg), BINP (0.04 mmol, 25 mg), NaOtBu (1.4 mmol, 134 mg), and toluene (0.11 M with 3-bromopyridine, 9 ml) is heated to 70°C under nitrogen until 2 is consumed. The reaction is then cooled to room temperature, and taken up in 10 ml diethyl ether, washed 3 times with 10 ml saturated brine, dried over $MgSO_4$, and condensed in *vacuo* . The crude product is purified by flash chromatography to afford 3.

<u>7-(3,4-dichlorophenyl)-2-methyl-3-(3-aminoheptane)-(4,6-c)-imidazo-pyridine (4):</u>

A mixture of 3 (0.14 mmol) and triethyl orthoacetate (0.5 ml) is heated at 110°C in reaction vial over night. The mixture is dissolved in ethyl acetate (10 ml), washed with water, dried over sodium sulfate and concentrated in *vacuo*. The crude product is purified by flash chromatography to afford 4.

EXAMPLE 6

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (II)



<u>4-(2,4-dichlorophenyl)-1-(4-hydroxy1-butyl)-imidazo-(4,5-c)-pyridine (2):</u>

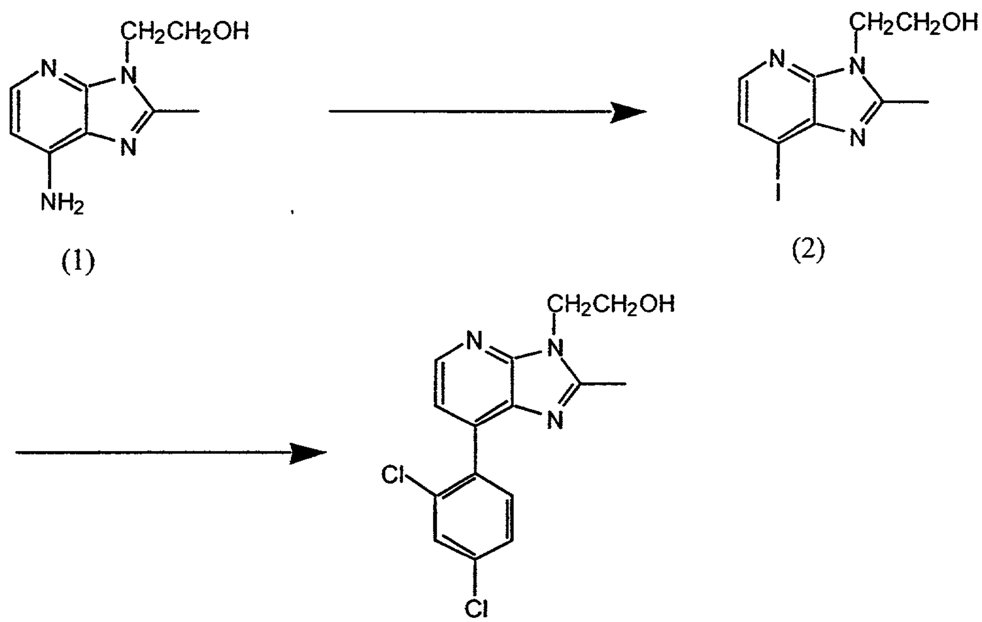
A solution of (1)[1] (1.0 g, 4.43 mmol), $Pd(PPh_3)_4$ (0.23 g, 0.22 mmol) and aqueous solution of Na_2CO_3 (6.6 ml, 2M) in toluene (25 ml) is added to a solution of dichlorobenzene boronic acid (1.74 g, 8.86 mmol) in ethanol (7 ml). The mixture is

refluxed for 14 h., diluted with ethyl acetate and washed with saturated NH_4Cl solution. The organic layer is dried over sodium sulfate and concentrated in *vacuo*. The residue is chromatographed on silica gel to give 2.

(Ronald T. Borchardt et al., *J. Med. Chem.* *28*:467-471, 1985)

EXAMPLE 7

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (II)



(3)

2-Iodo-7-methyl-6-ethanol-(4,5-b)-imidazo-pyridine (2):

To a solution of (1)[1] (1.12 mmol) in 2N HCl (8 ml) is added at ice bath temperature sodium nitrite (84 mg, 1.2 mmol) in water (4 ml). The mixture is stirred at ice bath temperature for 15 min. and added dropwise to a solution of potassium iodide (340 mg, 2.08 mmol) in water (4 ml). The reaction is heated to 60°C for 1h. The

solution is basified with 2M NaOH and extracted with ethyl acetate, washed with brine and concentrated in vacuo. the residue is purified by chromatography on silica gel to give the product 2.

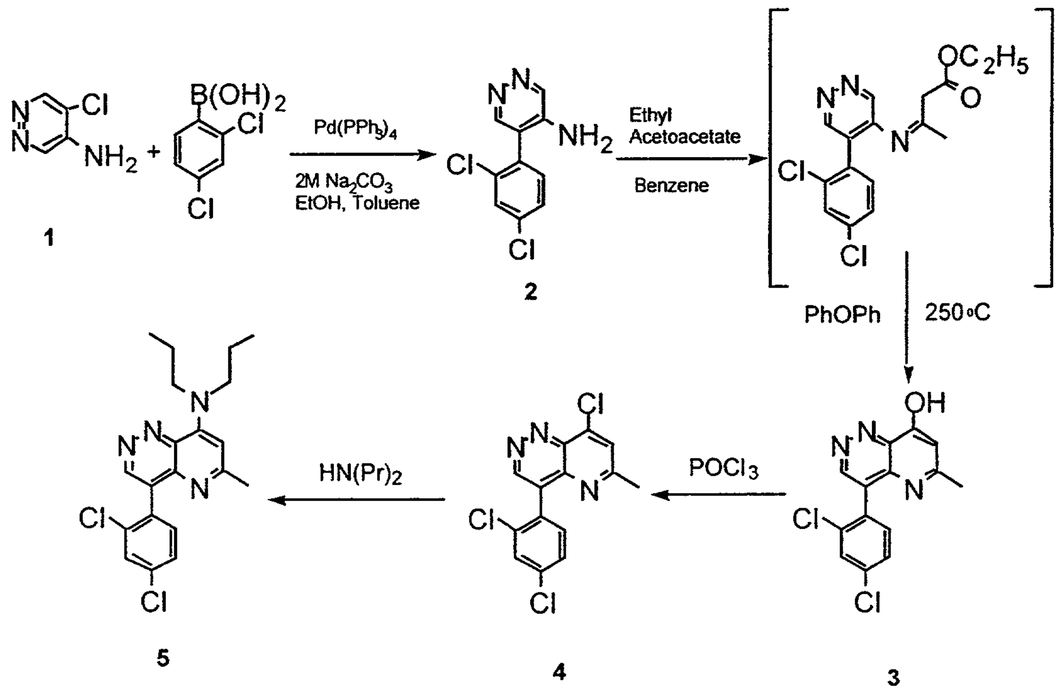
2-(2,4-dichlorophenyl)-7-methyl-6-ethanol-(4,5-b)-imidazo-pyridine (3):

A solution of (2) (4.43 mmol), $Pd(PPh_3)_4$ (0.22 mmol) and aqueous solution of Na_2CO_3 (6.6 ml, 2M) in toluene (25 ml) is added to a solution of dichlorobenzene boronic acid (8.86 mmol) in ethanol (7 ml). The mixture is refluxed for 14 h., diluted with ethyl acetate and washed with saturated NH_4Cl solution. The organic layer is dried over sodium sulfate and concentrated in *vacuo*. The residue is chromatographed on silica gel to give 3.

(Ramsden, Christopher A. J., *Chem. Soc. Perkin Trans. 21*:2789-2812, 1992).

EXAMPLE 8

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (III)



4-Amino-5-(3,4-dichlorophenyl)pyridizine (2)

To a solution of 4-amino-5-choloropyridizine (1) (3.25 g, 25 mmol) in toluene (100 mL) under argon add tetrakis(triphenylphosphine)palladium(0) (2.9g. 2.5 mmol), 2M aqueous sodium carbonate (35 mL) (degassed) and a solution of dicholorobenzeneboronic acid (5.3 g, 27 mmol) in ethanol (35 ml) (degassed). Heat the resulting two phase mixture for 16 hours at reflux under argon. Dilute the reaction with ethyl acetate (50 mL) and wash the organic layer with saturated ammonium chloride, dry (Na_2SO_4) and evaporate to dryness in vacuo. Purify by flash chromatography and isolate the desired product 2 by combining the appropriate fractions and evaporating to dryness.

2-Methyl-4-hydroxy-8-(3,4-dichlorophenyl)pyrido[2,3-c]pyridazine (3)

Reflux a solution of 4-amino-5-(3,4-dichlorophenyl)pyridazine (2) (4.8 g, 20 mmol), ethyl acetoacetate (2.7 g, 20 mmol) and p-toluenesulfonic acid monohydrate (20 mg) in benzene (100 mL) for 30 minutes. Cool the reaction mixture to ambient temperature and purify the intermediate product by flash chromatography. Add a solution of the intermediate (3.5 g, 10 mmol) in 5 mL of diphenyl ether to 10 mL of diphenyl ether at 240°C and reflux for 5 minutes. Cool the reacton and collect the resulting solid by filtration. Wash the product 3 with ether and dry .

2-Methyl-4-chloro-8-(3,4-dichlorophenyl)pyrido[2,3-c]pyridazine (4)

Reflux a mixture of 2-methyl-4-hydroxy-8-(3,4-dichlorophenyl)pyrido[2,3-c]pyridazine (3) (3.0 g, 10 mmol) in phosphorous oxychloride (10 mL) for 2 hrs. Cool the reaction mixture, pour onto cracked ice and neutralize the solution with 1N NaOH. Extract the solution with ethyl acetate (2 x 100 mL) and wash the combined organic layers with brine. Dry the solution (Na_2SO_4) and evaporate in vacuo to obtain 4.

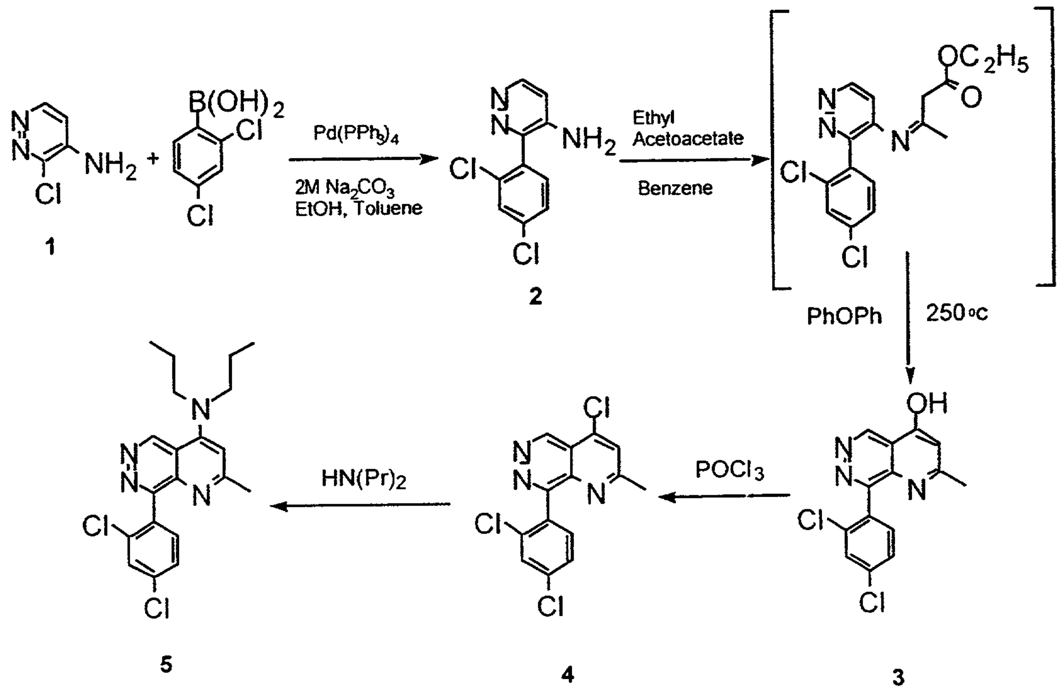
2-Methyl-4-dipropylamino-8-(3,4-dichlorophenyl)pyrido[2,3-c]pyridazine (5)

Heat a mixture of 2-methyl-4-chloro-8-(3,4-dichlorophenyl)pyrido[2,3-c]pyridazine (4) (1.0 g, 3.1 mmol) and p-toluenesulfonic acid (1.6 g) in 5 mL of di-n-propylamine in a sealed tube at 180°C for 48 hours. Cool the reaction mixture to ambient temperature and partition between ethyl acetate and water. Wash the organic layer with water and dry over $MgSO_4$. Evaporate the dried solution and purify the desired product 5 by flash chromatography.

(T. Kuraishi, R. N. Castle, *J. Heterocyclic Chem.* *1*:42, 1964)

EXAMPLE 9

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (III)



3-(3,4-Dichlorophenyl)-4-aminopyridizine (2)

To a solution of 4-amino-3-choloropyridizine (1) (6.5 g, 50 mmol) in toluene (200 mL) under argon add tetrakis(triphenylphosphine)palladium(0) (5.8g. 5 mmol), 2M aqueous sodium carbonate (75 mL) (degassed) and a solution of dicholorobenzeneboronic acid (10.6 g, 54 mmol) in degassed ethanol (75 ml). Heat the two phase mixture for 16 hours at reflux under argon. Dilute the reaction with ethyl acetate (100 mL) and wash the organic layer with saturated ammonium chloride, dry (Na_2SO_4) and evaporate to dryness in vacuo. Purify by flash chromatography and isolate the desired product 2 by combining the appropriate fractions and evaporating them to dryness.

2-Methyl-4-hydroxy-8-(3,4-dichlorophenyl)pyrido[2,3-d]pyridazine (3)

Reflux a solution of 3-(3,4-dichlorophenyl)-4-aminopyridizine (2) (7.2 g, 30 mmol), ethyl acetoacetate (4.0 g, 30 mmol) and of p-toluenesulfonic acid monohydrate (30 mg) in benzene (150 mL) for 30 minutes. Cool the reaction mixture to ambient temperature and purify the intermediate product by flash chromatography. Add a solution of the intermediate (5.25 g, 15 mmol) in 10 mL of diphenylether to 15 mL of diphenylether at 240°C and reflux for 5 minutes. Cool the reacton and collect the resulting solid (3) by filtration. Wash the product 3 with ether and dry .

2-Methyl-4-chloro-8-(3,4-dichlorophenyl)pyrido[2,3-d]pyridazine (4)

Reflux a mixture of 2-methyl-4-hydroxy-8-(3,4-dichlorophenyl)pyrido[2,3-d]pyridazine (3) (4.5 g, 15 mmol) in phosphorous oxychloride (15 mL) for 2 hrs. Cool the reaction mixture, pour onto cracked ice and neutralize the solution with 1N NaOH. Extract the solution with ethyl acetate (2 x 100 mL) and wash the combined organic layers with brine. Dry the solution (Na_2SO_4) and evaporate in vacuo to obtain 4.

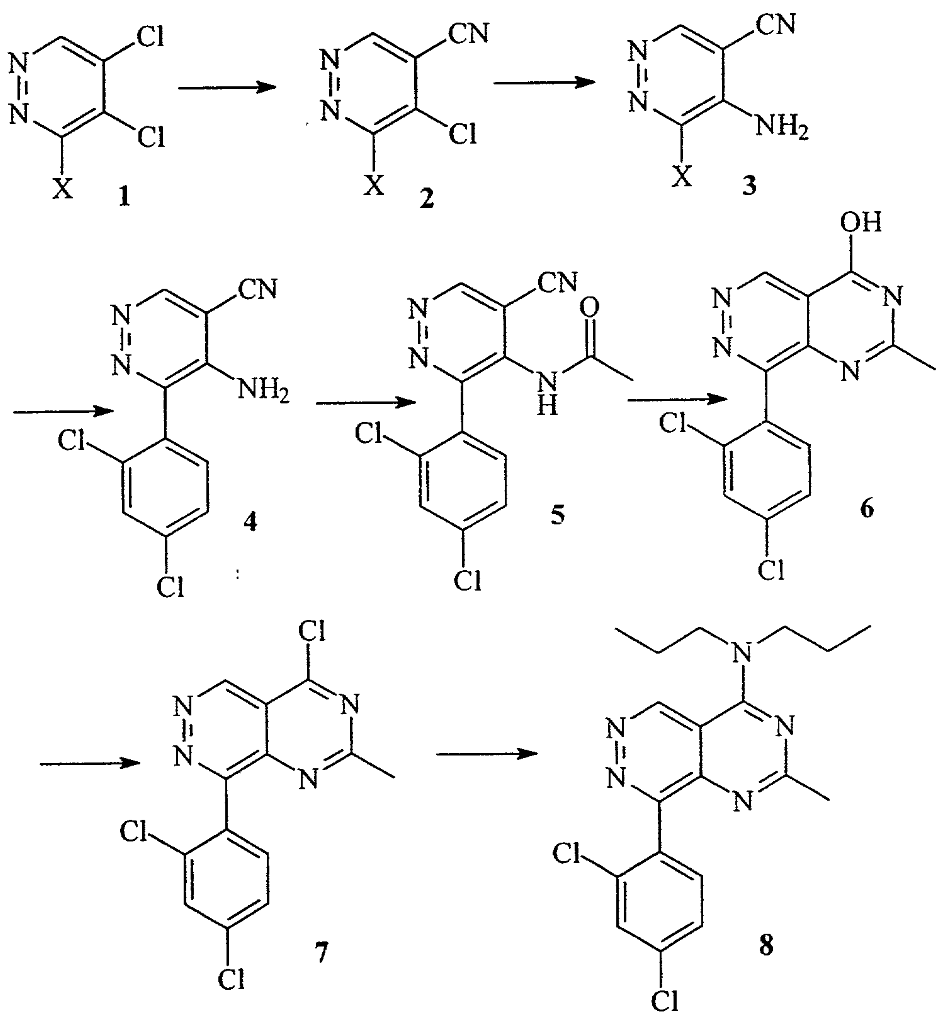
2-Methyl-4-dipropylamino-8-(3,4-dichlorophenyl)pyrido[2,3-d]pyridazine (5)

Heat a mixture of 2-methyl-4-chloro-8-(3,4-dichlorophenyl)pyrido[2,3-d]pyridazine (4) (2.0 g, 6.2 mmol) and p-toluenesulfonic acid (3.2 g) in 10 mL of di-n-propylamine in a sealed tube at 180°C for 48 hours. Cool the reaction mixture to ambient temperature and partition the reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry over $MgSO_4$. Evaporate the dried solution and purify the product 5 by flash chromatography.

(D. E. Kiinge, H. C. van der Plas, G. Geurtsen, A. Koudijs, *Recl. Trav. Chim. (Pays-Bas) 93*:236, 1974)

EXAMPLE 10

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (III)



Compound 2:

Compound 2 is synthesized by refluxing a mixture of compound 1 (X= Cl, Br) 10.0 mmol), sodium cyanide (3.0 gms), aluminium oxide (2.8 gms) and tetrakistriphenylphosphine palladium (1.0 mmol) in toluene (100 ml) under a nitrogen atmosphere. After refluxing overnight, the mixture is cooled and partitioned between

ethyl acetate and water. The organic layers are dried over sodium sulfate and the solvent removed under vacuum. The compound is purified by, for example, flash chromatography eluting with a mixture of ethyl acetate and hexane.

<u>Compound 3:</u>

Compound 3 is synthesized by heating 2 (9.0 mmol) in a solution of ammonia in a Parr bomb at 150°C. The solution is concentrated on rotary evaporator and the crude solid purified by for example recrystallization or flash chromatography eluting with hexanes:ethyl acetate to give product 3.

<u>Synthesis of Compound 4:</u>

A mixture of 3 (8 mmol), and a suitable boronic acid such as: 2,4-Dichlorobenzene boronic acid (1.8 gms, 9.5 mmol), tetrakis(triphenylphospine)palladium (0.95 mmol), and potassium carbonate (2.12 gms) is partitioned in a mixture of toluene, ethanol, and water (35 ml. 10 ml, 10 ml respectively). The mixture is refluxed under a nitrogen atmosphere for 18 hrs. After reflux, the solution is quenched with 50 ml saturated ammonium chloride and extracted with ethyl acetate. The organic layers are combined, dried over sodium sulfate and concentrated to yield oil. The crude oil is purified with flash chromatography eluting with, for example, hexanes:ethyl acetate to yield 4.

<u>Compound 6:</u>

Compound 5 is synthesized by heating 4 (2.38 gms, 9.0 mmol) in acetic anhydride (10 mmole) and acetic acid (20 mL) for 2 hours. After reflux, the resulting solution of 5 is concentrated down on rotary evaporator and the crude solid is dissolved up in phosphoric acid (85%, 10 ml). The solution is refluxed for 0.5 hours and poured over ice. A solid precipitates and is collected by filtration to leave compound 6.

<u>Compound 7:</u>

Compound 6 (10 mmole) and 3 mL of $POCl_3$ are mixed and heated to 100°C until most of the starting material is consumed, then allowed to cool to r.t., and

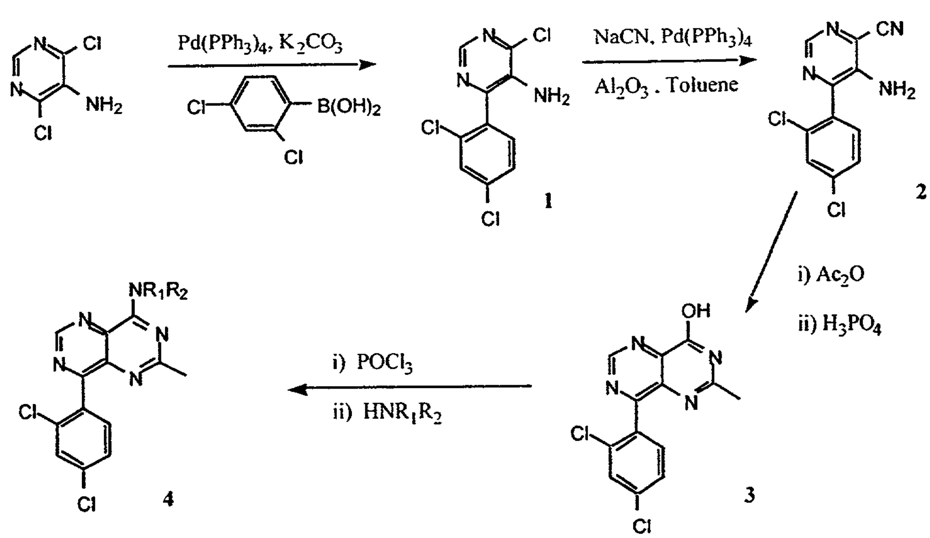
poured into 5% $NaHCO_3$. This is extracted with EtOAc, the organic phase is ished with brine, dried and concentrated. The product is purified by flash chromatography (SiO_2) using, for example, ethyl acetate/hexane, to give title compound.

2-Methyl-4-(dipropylamino)-8-(2,4-dichlorophenyl)pyridazino[4,5-d]pyrimidine (8):

Compound 7 (10 mmoles) and a secondary amine such as dipropylamine (100 mmoles) and 75 mL acetonitrile are refluxed for several hours until most of the chloro derivative is consumed. The reaction is poured into water and extracted with ethyl acetate. The organic phase is ished with water, then brine, dried ($MgSO_4$) and concentrated. The resulting residue is purified by flash chromatography (SiO_2) using, for example, ethyl acetate/hexane, to give title compound.

EXAMPLE 11

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (IV)



Synthesis of Compound 1

A mixture of 5-Amino-4,6-dichloropyrimidine (1.3 gms, 7.92 mmol), 2,4-Dichlorobenzene boranic acid (1.8 gms, 9.5 mmol), tetrakis triphenylphospine palladium (0.95 mmol), and potassium carbonate (2.12 gms) was partitioned in a mixture of toluene, ethanol, and water (35 ml, 10 ml, 10 ml respectively). The mixture was refluxed under a nitrogen atmosphere for 18 hrs. After reflux, the solution was quenched with 50 ml saturated ammonium chloride and extracted with ethyl acetate (2x300 ml). The organic layers were combined, dried over sodium sulfate and concentrated to yield oil. The crude oil was purified with flash chromatography eluting with hexanes:ethyl acetate (5:1) to yield yellow solid. 40% yield. δ 4.15 (br, 2H, NH), 7.34 (m, 2H, Ar), 7.53 (s, 1H, Ar), 8.43 (s, 1H, Ar(pyrmidine))ppm.

Compound 2

Compound 2 is synthesized by refluxing a mixture of compound 1 (2.73 gms, 10.0 mmol), sodium cyanide (3.0 gms), aluminium oxide (2.8 gms) and tetrakistriphenylphosphine palladium (1.0 mmol) in toluene (100 ml) under a nitrogen atmosphere. After refluxing overnight, the mixture is cooled and partitioned between ethyl acetate and water. The organic layers are dried over sodium sulfate and all solvent removed. The compound is purified by flash chromatography eluting with a hexane:ethyl acetate mixture.

Compound 3

Compound 3 is synthesized by refluxing 2 (2.38 gms, 9.0 mmol) in acetic anhydride (20 ml) for 2 hours. After reflux, the solution is concentrated down on rotary evaporator and the crude solid is dissolved up in phosphoric acid (85 %, 10 ml). The solution is refluxed for 0.5 hours and poured over 200 ml of ice. A white solid crashes out of the solution and is collected by filtration to leave pure compound 3.

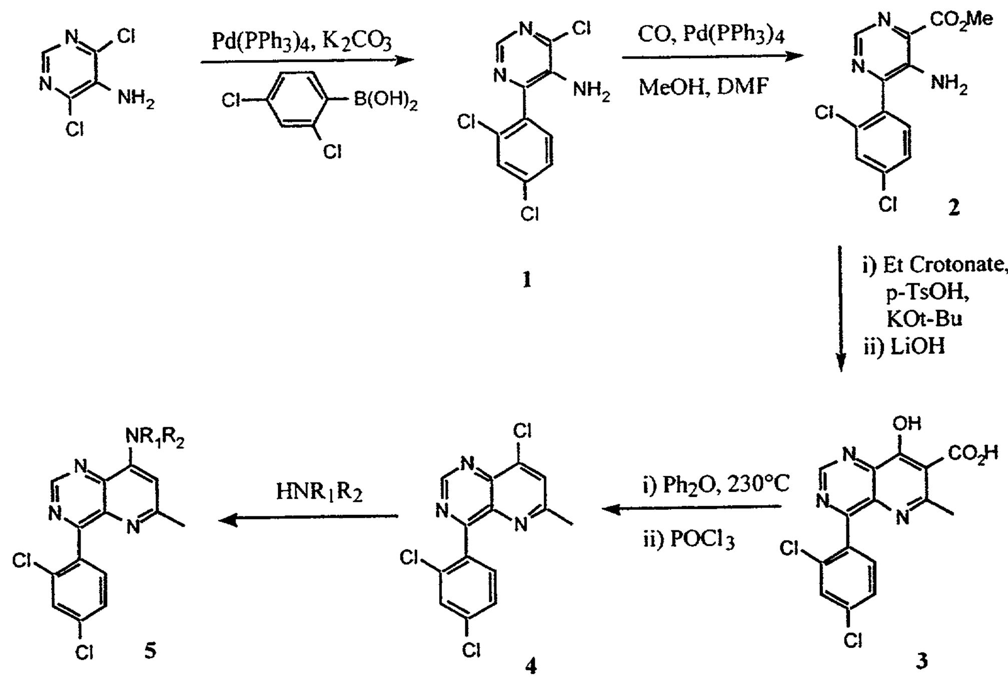
2-Methyl-4-(dipropylamino)-8-(2,4-dichlorophenyl)pyrimidino[5,4-d]pyrimidine (4)

A suspension of compound 3 (2.44 gms, 8.0 mmol) in phosphorous oxychloride (5 ml) is refluxed for one hour. After reflux, the dark solution is

concentrated on high vacuum pump to yield a crude dark solid. This crude solid is then dissolved up in 20 ml acetonitrile and refluxed in the presence of excess dipropyl amine. After two hours, the reaction is stopped and the solution is partitioned between ethyl acetate and sodium bicarbonate solution. The organic layers are separated and dried over sodium sulfate. All solvent is removed and the compound is purified by flash chromatography by eluting with hexanes:ether.

EXAMPLE 12

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (IV)



Synthesis of Compound 1

A mixture of 5-Amino-4,6-dichloropyrimidine (1.3 gms, 7.92 mmol), 2,4-Dichlorobenzene boranic acid (1.8 gms, 9.5 mmol), tetrakis triphenylphospine palladium (0.95 mmol), and potassium carbonate (2.12 gms) was partitioned in a

mixture of toluene, ethanol, and water (35 ml, 10 ml, 10 ml respectively). The mixture was refluxed under a nitrogen atmosphere for 18 hrs. After reflux, the solution was quenched with 50 ml saturated ammonium chloride and extracted with ethyl acetate (2x300 ml). The organic layers were combined, dried over sodium sulfate and concentrated to yield oil. The crude oil was purified with flash chromatography eluting with hexanes:ethyl acetate (5:1) to yield yellow solid. 40% yield. d 4.15 (br, 2H, NH), 7.34 (m, 2H, Ar), 7.53 (s, 1H, Ar), 8.43 (s, 1H, Ar(pyrmidine))ppm.

<u>Compound 2</u>

Compound 2 is synthesized by heating a mixture of compound 1 (2.73 gms, 10.0 mmol), and tetrakistriphenylphosphine palladium (1.0 mmol) in a methanol and DMF mixture under a carbon monoxide atmosphere (50 psi) in a Parr bomb at 100°C for 12 hours. The mixture is cooled and partitioned between ethyl acetate and water. The organic layers are dried over sodium sulfate and all solvent removed. The compound is purified by flash chromatography eluting with a hexane:ethyl acetate mixture.

<u>Compound 3</u>

A solution of 2 (1 gm, 3.36 mmol) and ethoxy crotonate (1.5g, 5.2 mmole) and 75 mg p-toluenesulfonic acid monohydrate in 50 mL xylene is stirred and heated to reflux under N_2. Solvent (25 mL) is removed by slow distillation over 1 h. The solution is allowed to cool to r.t. and a solution of potassium t-butoxide (570 mg, 5.1 mmole) in 12 mLs of absolute ethanol is added to the mixture. This mixture is heated to 80°C for 2 h. This is allowed to cool to r.t., and treated with 0.6 mL AcOH, then concentrated to dryness. The residue is suspended in EtOAc stirred, filtered and washed to remove all the product from the KOAc. The filtrate is concentrated to a small volume andused crude. A solution of the crude mixture, ethyl ester (1.7 g, 4.8 mmole) and 17.5 mL of 1 M LiOH in 10 mL ethanol is stirred and heated to reflux under N_2 for 16 h. The solution is then allowed to cool to r.t. and poured into a mixture of 15 mLs of 1 M hydrochloric acid in 100 mLs of water. This solution is extracted with EtOAc,

the organic phase washed with brine, dried and concentrated to give the title compound 3.

Compound 4

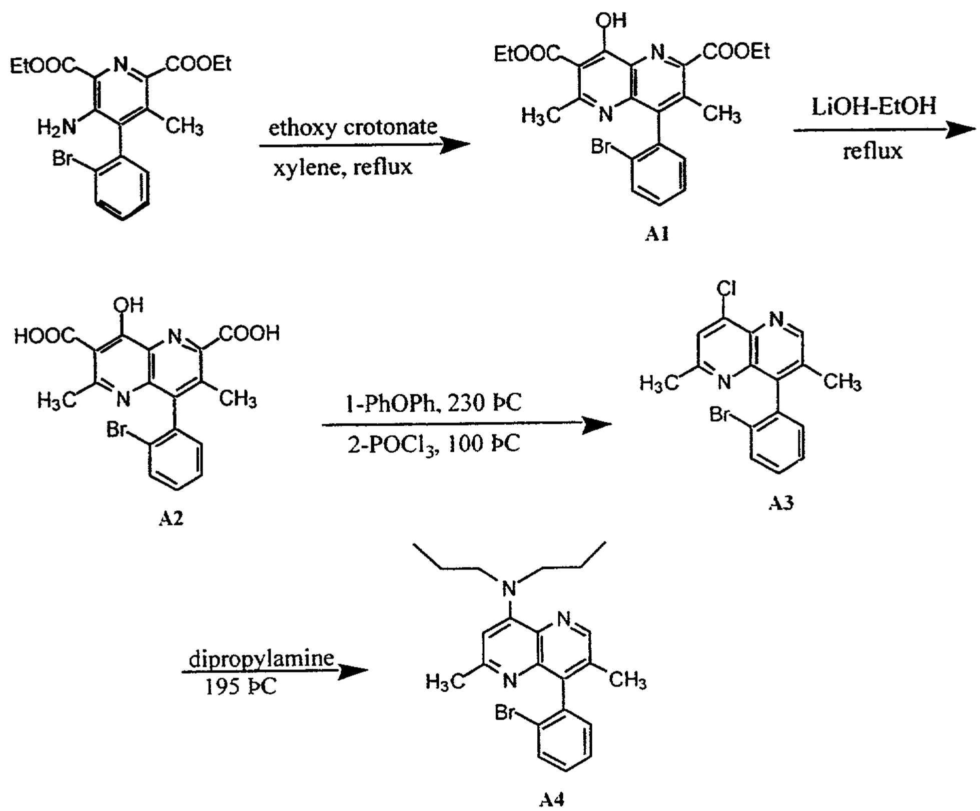
A solution of 3 (400 mg, 1.2 mmole) in 0.4 mL diphenyl ether is stirred and heated to 230°C for 1.5 h. The solution is then allowed to cool to r.t and 0.8 mL of $POCl_3$ added. This mixture is heated to 100°C for 2 h, then allowed to cool to r.t., and poured into 5% $NaHCO_3$. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by flash chromatography (SiO_2) using 0 to 10% ether/hexane, to give compound 4.

2-Methyl-4-(dipropylamino)-8-(2,4-dichlorophenyl)pyrido[3,2-d]pyrimidine (5, R1=R2= nPr)

A mixture of 4 (10 mg), p-toluenesulfonic acid (20 mg) and dipropylamine (50 ml) is stirred and heated to 195°C in a sealed tube for 1.5 h. The solution is then allowed to cool to r.t., and dissolved in a mixture of water and EtOAc. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by prep. TLC (SiO_2) using ethyl acetate/hexane.

EXAMPLE 13

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (V)



2,7-dimethyl-3,6-dicarboxy-4-hydroxy-8-(2'-bromophenyl)-1,5-naphthyridine, ethyl ester (A1):

A solution of 2,6-carboxy-3-amino-4-(2'-bromophenyl)-5-methyl pyridine, ethyl ester[1] (2.11 g, 5.2 mmole), ethoxy crotonate (1.0 equivalent) and p-toluenesulfonic acid monohydrate (75 mg) in xylene (50 ml) is stirred and heated to reflux under N_2. Solvent (25 ml) is removed by slow distillation over 1 h. The solution is allowed to cool to r.t. and a solution of potassium t-butoxide (570 mg, 5.1 mmole) in absolute ethanol (12 ml) is added. This mixture is heated to 80°C for 2 h. This is allowed to cool to r.t., treated with AcOH (0.6 ml) then concentrated to dryness. The residue is suspended in EtOAc stirred, filtered and washed to remove all the product

from the KOAc. The filtrate is concentrated to a small volume and treated with ethyl ether to precipitate the product A1.

2,7-dimethyl-3,6-carboxy-6-hydroxy-8-(2'-bromophenyl)-1,5-naphthyridine (A2):

A solution 2,7-dimethyl-3,6-dicarboxy-4-hydroxy-8-(2'-bromophenyl)-1,5-naphthyridine, ethyl ester (2.21 g, 4.8 mmole) and LiOH (17.5 ml, 1 M) in ethanol (10 ml) is stirred and heated to reflux under N_2 for 16 h. The solution is allowed to cool to r.t. then poured into a mixture of hydrochloric acid (15 ml, 1 M) in water (100 ml). This is extracted with EtOAc, the organic phase is washed with brine, dried and concentrated to give the title compound. This is used directly in the next step.

2,7-methyl-4-chloro-8-(2'-bromophenyl)-1,5-naphthyridine (A3):

A solution of 2,7-dimethyl-3,6-carboxy-6-hydroxy-8-(2'-bromophenyl)-1,5-naphthyridine (0.5 g, 1.2 mmole) in diphenyl ether (0.4 ml) is stirred and heated to 230°C for 1.5 h. The solution is allowed to cool to r.t and $POCl_3$ (0.8 ml) is added. This mixture is heated to 100°C for 2 h, then allowed to cool to r.t., and poured into 5% $NaHCO_3$. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by flash chromatography on silica gel to give the title compound.

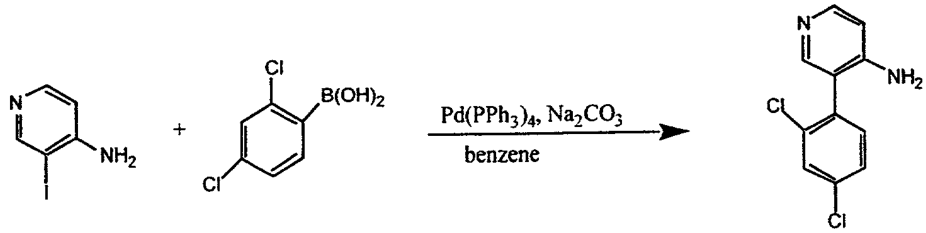
2,7-dimethyl-4-dipropylamino-8-(2'-bromophenyl)-1,5-naphthyridine (A4):

A mixture of 2,7-methyl-4-chloro-8-(2'-bromophenyl)-1,5-naphthyridine (10 mg, 0.02 mmole), p-toluenesulfonic acid (20 mg) and dipropylamine (50 μl) is stirred and heated to 195°C for 1.5 h. The solution is allowed to cool to r.t., then dissolved in a mixture of water and EtOAc. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by prep. TLC (SiO_2) using ethyl acetate/hexane, to give the product.

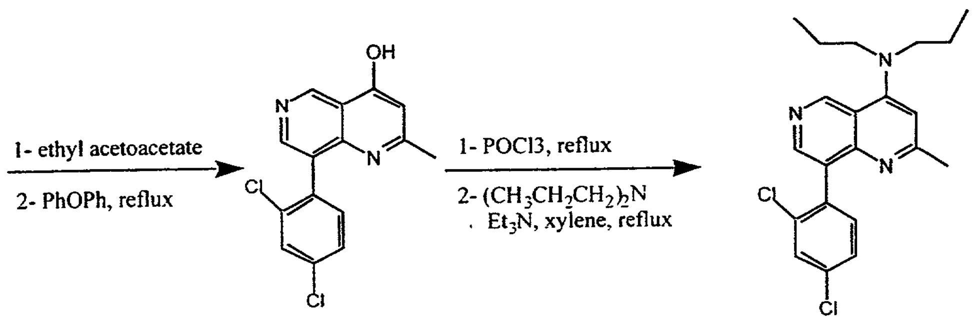
(Dale L. Boger, Steven R. Duff, James S. Panek, Masami Yasuda, *J. Org. Chem.* *50*:5782-5789, 1985)

EXAMPLE 14

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (V)



B1



B2 **B3**

<u>4-amino-3-(2',4'-dichlorophenyl)-pyridine (B1):</u>

A solution of 3-iodo 4-amino pyridine[1] (2.55 g, 11.6 mmole), $Pd(PPh_3)_4$ (0.67 g, 0.58 mmole) and aqueous solution of Na_2CO_3 (17.4 ml, 2M) in toluene (60 ml) is added to a solution of dichlorobenzene boronic acid (4.56 g, 23.2 mmole) in ethanol (17.6 ml). The mixture is heated at reflux for 14 h., diluted with ethyl acetate and washed with saturated NH_4Cl solution. The organic layer is dried over sodium sulfate and concentrated in *vacuo*, The residue is chromatographed on silica gel to give <u>B1</u>.

<u>2-methyl 4-hydroxy-8-(2',4'-dichlorophenyl)1,6-naphthyridine(B2):</u>

A solution of <u>B1</u> (2.41 g, 10.1 mmole), ethyl acetate (1.31 g, 10.1 mmole) and p-toluenesulfonic acid (70 mg) in benzene (50 ml) is heated at reflux for 2h. during which 10 ml of solvent is removed by distillation. After evaporation, the

residue is added to boiling diphenyl ether (10 ml) and heating is continued for 1h; then the solution is cooled and poured into high boiling petroleum ether with vigorous stirring. The resulting solid formed is filtered off and washed with diethyl ether to give the product B2.

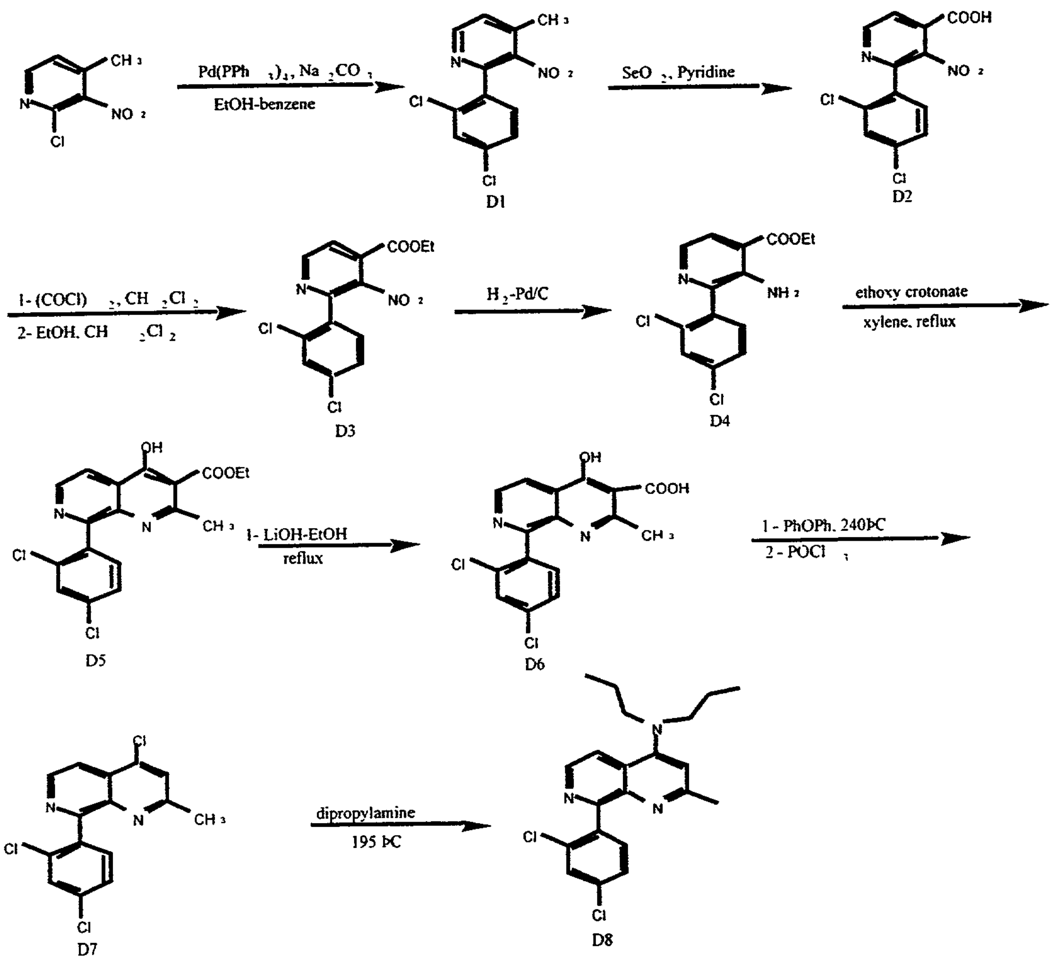
2-methyl 4-dipropylamino-8-(2',4'-dichlorophenyl)-1,6-naphthyridine (B3):

A mixture of B2 (0.10 mg, 0.32 mmole) and phosphorous oxychloride (0.5 ml) is heated at reflux for 6h. Excess reagent is removed in *vacuo* and the residual compound is treated with dipropyl amine (100 mg) and triethylamine (100 mg) in xylene (2 ml) and the mixture is refluxed for 14 h. The solution is poured into ethyl acetate and washed with dilute bicarbonate solution, the organic layer is dried and the solvent removed in *vacuo*. The residue is chromatographed on silica gel to afford B3.

(L. Estel, F. Marsais, G. Queguiner, *J. Org. Chem. 53*:2740-2744, 1988)

EXAMPLE 15

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (V)



2-(2',4'-dichloropheyl)-3-nitro-4-methyl pyridine (D1):

A solution of 2-chloro-3-nitro-4-methyl pyridine (4.0 g, 23.2 mmole), $Pd(PPh_3)_4$ (1.34 g, 1.16 mmole) and aqueous solution of Na_2CO_3 (34.8 ml, 2M) in toluene (120 ml) is added to a solution of dichlorobenzene boronic acid (9.12 g, 46.4 mmole) in ethanol (34 ml). The mixture is refluxed for 14 h., diluted with ethyl acetate and washed with saturated NH_4Cl solution. The organic layer is dried over sodium

sulfate and concentrated in *vacuo*. The residue is chromatographed on silica gel to give D1.

2-(2',4'-dichlorophenyl)-3-nitro-4-carboxy pyridine (D2):

A mixture of 2-(2',4'-dichlorophenyl)-3-nitro-4-methyl pyridine (2.5g, 8.82 mmole) and SeO_2 (0.97, 8.82 mmole) in pyridine is heated at 117°C for 6h. The reaction is cooled to r.t., filtered and the solvent is evaporated under high vacuum. Compound D2 is used directly in the next step.

2-(2',4'-dichlorophenyl)-3-nitro-4-carboxy pyridine, ethyl ester (D3):

To a suspension of 2-(2',4'-dichlorophenyl)-3-nitro-4-carboxy pyridine (2.0 g, 6.38 mmole) and $(COCl)_2$ (0.75 g, 7.65 mmole) in CH_2Cl_2 (10 ml) is added a drop of DMF and the mixture is stirred at r.t. for 1h. After evaporation of solvent and excess reagent the solid is dissolved in CH_2Cl_2 (10 ml) and treated with EtOH (5 ml) and $NaHCO_3$ (1.0 g). the mixture is stirred at r.t. for 1h. filtered and concentrated in *vacuo* to give compound D3.

2-(2',4'-dichlorophenyl)-3-amino-4-carboxy pyridine, ethyl ester (D4):

A mixture of 2-(2',4'-dichlorophenyl)-3-nitro-4-carboxy pyridine, ethyl ester (1.8 g, 5.27 mmole) and 10% Pd/C in EtOH is shaken at r.t. under 40 psi of hydrogen for 3h. The catalyst is removed by filtration through celite and the solution is concentrated in vacuo to give compound D4.

2-methyl-3-carboxy-4-hydroxy-8-(2',4'-dichlorophenyl)-1,7-naphthyridine, ethyl ester (D5):

A solution of 2-(2',4'-dichlorophenyl)-3-amino-4-carboxy pyridine, ethyl ester (1.61 g, 5.2 mmole), ethoxy crotonate (g, 5.2 mmole) and p-toluenesulfonic acid monohydrate (75 mg) in xylene (50 ml) is stirred and heated to reflux under N_2. Solvent (25 ml) is removed by slow distillation over 1 h. The solution is allowed to cool to r.t. and a solution of potassium t-butoxide (570 mg, 5.1 mmole) in absolute ethanol (12 ml) is added. This mixture is heated to 80°C for 2 h. This is allowed to cool

to r.t., treated with AcOH (0.6 ml) then concentrated to dryness. The residue is suspended in EtOAc stirred, filtered and washed to remove all the product from the KOAc. The filtrate is concentrated to a small volume and treated with ethyl ether to precipitate the product D5.

2-methyl-3-carboxy-4-hydroxy-8-(2',4'-dichlorophenyl)-1,7-naphthyridine (D6):

A 2-methyl-3-carboxy-4-hydroxy-8-(2',4'-dichlorophenyl)-1,7-naphthyridine, ethyl ester (1.80 g, 4.8 mmole) and LiOH (17.5 ml, 1 M) in ethanol (10 ml) is stirred and heated to reflux under N_2 for 16 h. The solution is allowed to cool to r.t. then poured into a mixture of hydrochloric acid (15 ml, 1 M) in water (100 ml). This is extracted with EtOAc, the organic phase is washed with brine, dried and concentrated to give the title compound. This is used directly in the next step.

2-methyl-4-chloro-8-(2',4'-dichlorophenyl)-1,7-naphthyridine (D7):

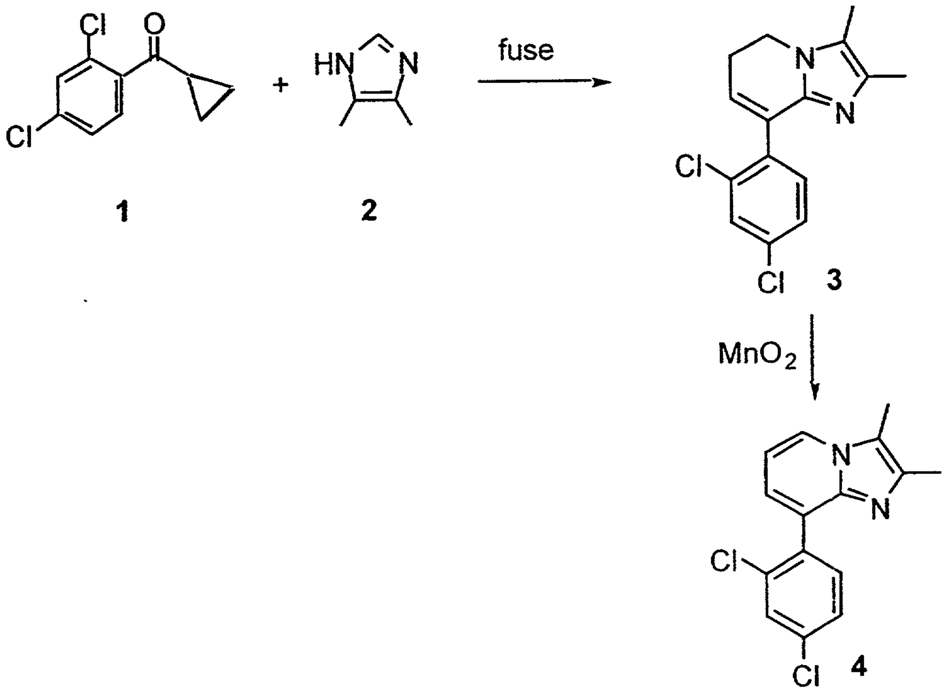
A solution of 2-methyl-3-carboxy-4-hydroxy-8-(2',4'-dichlorophenyl)-1,7-naphthyridine (0.41 g, 1.2 mmole) in diphenyl ether (0.4 ml) is stirred and heated to 230°C for 1.5 h. The solution is allowed to cool to rt. and $POCl_3$ (0.8 ml) is added. This mixture is heated to 100°C for 2 h, then allowed to cool to r.t., and poured into 5% $NaHCO_3$. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by flash chromatography on silica gel to give the title compound D7.

2-dimethyl-4-dipropylamino-8-(2',4'-dichlorophenyl)-1,7-naphthyridine (D8):

A mixture of 2-methyl-4-chloro-8-(2',4'-dichlorophenyl)-1,7-naphthyridine (6.47 mg, 0.02 mmole), p-toluenesulfonic acid (20 mg) and dipropylamine (50 µl) is stirred and heated to 195°C for 1.5 h. The solution is allowed to cool to r.t., then dissolved in a mixture of water and EtOAc. This is extracted with EtOAc, the organic phase washed with brine, dried and concentrated. The product is purified by prep. TLC (SiO_2) using ethyl acetate/hexane, to give the product D8.

EXAMPLE 16

SYNTHESIS OF REPRESENTATIVE COMPOUNDS OF STRUCTURE (VI)



2,3-Dimethyl-8-(2,4-dichlorophenyl)-5,6-dihydroimidazo[1,2-a]pyridine (3)

Cyclopropyl 2,4-dichlorophenyl ketone (1) (54 g, 0.25 mol) and 4,5-dimethylimidazole (2) (90 g, 1 mol) are combined and heated at 200 to 210°C under nitrogen for 20 hours. The reaction is cooled and diluted with ethyl acetate (700 ml). The ethyl acetate solution is washed with saturated aq potassium carbonate (300 ml) and water (4 x 200 ml) and dried over sodium sulfate. The drying agent is removed by filtration and the solvent is removed in vacuo to provide 3.

2,3-Dimethyl-8-(2,4-dichlorophenyl)imidazo[1,2-a]pyridine (4)

2,3-Dimethyl-8-(2,4-dichlorophenyl)-5,6-dihydroimidazo[1,2-a]pyridine (3) (29.3 g, 0.1 mol) is dissolved in methylene chloride (1L) and activated manganese dioxide (120 g) is added. The mixture is heated at reflux for 16 hours with stirring. The

catalyst is removed by filtration of the reaction through a Celite pad and the filtrate is evaporated to a solid (4).

EXAMPLE 17

REPRESENTATIVE COMPOUNDS HAVING CRF RECEPTOR BINDING ACTIVITY

The compounds of this invention may be evaluated for binding activity to the CRF receptor by a standard radioligand binding assay as generally described by DeSouza et al. (*J. Neurosci.* 7:88-100, 1987). By utilizing various radiolabeled CRF ligands, the assay may be used to evaluate the binding activity of the compounds of the present invention with any CRF receptor subtype. Briefly, the binding assay involves the displacement of a radiolabeled CRF ligand from the CRF receptor.

More specifically, the binding assay is performed in 1.5 ml Eppendorf tubes using approximately 1 x 10^6 cells per tube stably transfected with human CRF receptors. Each tube receives about 0.1 ml of assay buffer (*e.g.*, Dulbecco's phosphate buffered saline, 10 mM magnesium chloride, 20 μM bacitracin) with or without unlabeled sauvagine, urotensin I or CRF (final concentration, 1 μM) to determine nonspecific binding, 0.1 ml of [^{125}I] tyrosine - ovine CRF (final concentration ~200 pM or approximately the K_D as determined by Scatchard analysis) and 0.1 ml of a membrane suspension of cells containing the CRF receptor. The mixture is incubated for 2 hours at 22°C followed by the separation of the bound and free radioligand by centrifugation. Following two washes of the pellets, the tubes are cut just above the pellet and monitored in a gamma counter for radioactivity at approximately 80% efficiency. All radioligand binding data may be analyzed using the non-linear least-square curve-fitting program LIGAND of Munson and Rodbard (*Anal. Biochem.* *107*:220, 1990).

EXAMPLE 18

CRF-STIMULATED ADENYLATE CYCLASE ACTIVITY

The compounds of the present invention may also be evaluated by various functional testing. For example, the compounds of the present invention may be screened for CRF-stimulated adenylate cyclase activity. An assay for the determination of CRF-stimulated adenylate cyclase activity may be performed as generally described by Battaglia et al. (*Synapse 1*:572, 1987), with modifications to adapt the assay to whole cell preparations.

More specifically, the standard assay mixture may contain the following in a final volume of 0.5 ml: 2 mM L-glutamine, 20 mM HEPES, and 1 mM IMBX in DMEM buffer. In stimulation studies, whole cells with the transfected CRF receptors are plated in 24-well plates and incubated for 1 h at 37°C with various concentrations of CRF-related and unrelated peptides in order to establish the pharmacological rank-order profile of the particular receptor subtype. Following the incubation, the media is aspirated, the wells rinsed once gently with fresh media, and the media aspirated. To determine the amount of intracellular cAMP, 300 µl of a solution of 95% ethanol and 20 mM aqueous hydrochloric acid is added to each well and the resulting suspensions are incubated at -20°C for 16 to 18 hours. The solution is removed into 1.5 ml Eppendorf tubes and the wells washed with an additional 200 µl of ethanol/aqueous hydrochloric acid and pooled with the first fraction. The samples are lyophilized and then resuspended with 500 µl sodium acetate buffer. The measurement of cAMP in the samples is performed using a single antibody kit from Biomedical Technologies Inc. (Stoughton, MA). For the functional assessment of the compounds, a single concentration of CRF or related peptides causing 80% stimulation of cAMP production is incubated along with various concentrations of competing compounds (10^{-12} to 10^{-6} M).

It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications

may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.